INTESTINAL HELMINTH INFECTIONS, DISTRIBUTIONS, AND ASSOCIATIONS WITH HEALTH PARAMETERS OF SPRING-MIGRATING FEMALE LESSER SCAUP IN THE UPPER MIDWEST

BY

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THESIS
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ABSTRACT

The continental breeding population of Lesser Scaup (*Aythya affinis*) reached a record low of 3.2 million in 2006 and has since remained below the goal of 6.3 million set by the North American Waterfowl Management Plan. Although many factors have been identified as possible contributors to the decline, the Spring Condition Hypothesis proposes reduced recruitment resulting from females in reduced condition as the most influential cause. Since 2002, infections with non-native trematodes transmitted by the invasive Faucet Snail (*Bithynia tentaculata*) have resulted in the deaths of tens of thousands of Lesser Scaup in the upper Midwest, USA during migrations. To determine if parasitic intestinal helminths were associated with reduced body condition and health, I collected 130 apparently healthy female Lesser Scaup and identified and enumerated helminths within their intestinal tracts. I also measured a suite of health parameters to identify associations between intestinal helminth infections and their sub-lethal effects on Lesser Scaup. Forty helminth taxa (20 trematodes, 14 cestodes, 4 nematodes, and 2 acanthocephalans) were identified, including one digenean (*Plenosoma minimum*) for the first time in Lesser Scaup and in the Midwest. Helminth species diversity and mean total helminth abundance were greatest in northern portions of the study region along the Mississippi River, and mean total helminth abundance was less in 2015 than in 2014. Host age and body size were not associated as factors contributing to helminth assemblage, which may be due in part to physiological changes experienced during migration. The intestinal helminth infracommunities of Lesser Scaup were found to vary across the four regions and two years of the study. Variations in helminth infracommunity structure were likely due to geophysical variation within the study area, prey item (intermediate host) diversity, and weather pattern variations that occurred between years thus affecting migration chronologies and prey abundance. Varying associations were detected between 11 health parameters and seven response variables of differing helminth metrics. Most notably were the relationships observed between helminth species diversity and body fat, albumin, glucose, and percentage of white blood cells that were monocytes. Total trematode abundance was negatively related to the plasma metabolite concentrations of non-esterified

fatty acids and albumin. Total nematode abundance showed a strong negative association with plasma bilirubin concentrations. The daily lipid dynamic index, a ratio of blood metabolites used as an index of foraging habitat quality, showed a negative association with total cestode abundance and *Sphaeridiotrema* spp. intensity. Cestode total abundance was also negatively associated with the heterophil:lymphocyte ratio, an indicator of long-term stress, but displayed a strong, positive relationship with plasma bilirubin concentrations. The introduced trematodes responsible for annual die-offs of Lesser Scaup in the upper Midwest displayed varying relationships with health parameters. While a positive relationship was detected between the introduced *Cyathocotyle bushiensis* and basophil white blood cells, negative associations were observed between *Sphaeridiotrema* spp. and bilirubin concentrations, packed cell volumes, and habitat quality variables. These biochemical and hematological measurements are important to physiological homeostasis and serve as indicators of condition at a critical period of the Lesser Scaup annual cycle. The associations between the health parameters, helminth metrics, and habitat quality measurements reported herein provide additional evidence for possible mechanisms underlying the Spring Condition Hypothesis in the upper Midwest, USA.

DEDICATION

For my amazing better half and beautiful wife, Jenna, who has followed me across all of Creation, while I have pursued this endeavor like a hound on a scent. Her enduring love and patience go unmatched on this earth. I could not have done this without you, and I am extremely thankful to have you as my wife and mother of our little girl, Cedar. Thank you both for sticking this out with me. I'm a very lucky man.

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CHAPTER 1: SPATIOTEMPORAL DISTIBUTIONS OF INTESTINAL HELMINTHS FROM FEMALE LESSER SCAUP DURING SPRING MIGRATION IN THE UPPER MIDWEST, USA

1.1 INTRODUCTION

Since approximately 2002, tens of thousands of waterbirds have been reported dying each year from infections of introduced trematodes (Digenea) during spring and fall migration events in the upper Midwest, USA, and no species has been harder hit than the Lesser Scaup (*Aythya affinis*) (Sauer et al. 2007; Herrmann and Sorensen 2011; Sandland et al. 2014). The continental breeding population of Lesser Scaup reached a record low of 3.2 million in 2006 and has since remained below the goal of 6.3 million set by the North American Waterfowl Management Plan (Austin et al. 2000; Afton and Anderson 2001). A myriad of factors, including habitat loss and degradation, climate change, predation, competition, invasive species, and contaminants, have been identified as stressors influencing Lesser Scaup condition and subsequent breeding success (Afton and Anderson 2001; Anteau and Afton 2004, 2006, 2008a; Austin et al. 2006; Strand et al. 2008; Drever et al. 2012). The Spring Condition Hypothesis suggests that the major contributor to the population decline is reduced recruitment resulting from female Lesser Scaup reaching their breeding grounds in poor condition (Anteau and Afton 2004, 2008a,b, 2011).

Previous research has linked poor body condition to quantity and quality of foods, but a related factor, parasitism, may also support the Spring Condition Hypothesis. However, no large-scale studies have examined the distribution and overall loads of parasites, including non-native trematodes, which occur in the upper Midwest, an important stopover region for spring-migrating Lesser Scaup. Parasitism is an important ecological factor influencing populations both directly and indirectly through diversion of energy from the host species (e.g., blood meal acquisition and initiation of an immune response respectively) (Wobeser 2008). However, parasites are often not considered to be significant regulators of wildlife populations by researchers unless infections result in mass mortalities (Hudson et al. 1998; Lafferty 2010).

Historically in Midwestern wetlands, Lesser Scaup diets included primarily amphipods (*Gammarus lacustris* and *Hyalella azteca*) during the spring and summer months; however, due to anthropogenic alterations to wetlands and increased runoff from agricultural fields, amphipods populations have declined throughout the Midwest (Anteau and Afton 2008a; Anteau et al. 2011). Declines in amphipod populations have caused Lesser Scaup diets to shift to the more abundant, albeit invasive, gastropod and bivalve species (e.g., Faucet Snail [*Bithynia tentaculata*] and Zebra Mussel [*Dreissena polymorpha*]) in the upper Midwest (Anteau and Afton 2006; Herrmann and Sorensen 2011; Karatayev et al. 2012). The Faucet Snail is native to many of the waterways of Eurasia and was most likely introduced into the Great Lakes during the late 1800's via cargo ship ballast waters (Mills et al. 1993). This snail serves as the first and second intermediate host for the introduced trematodes *Cyathocotyle bushiensis*, *Sphaeridiotrema pseudoglobulus*, and *Sphaeridiotrema globulus* which are considered responsible for the large scale mortality events of Lesser Scaup in the upper Midwest and elsewhere in the Faucet Snail's expanding range (Cole and Friend 1999; Sandland et al. 2014). Earlier publications may have mistakenly identified *S. pseudoglobulus* as the closely related *S. globulus* (McLaughlin et al. 1993), yet there is ongoing debate as to which species is most prevalent in water bodies associated with the upper Midwest (Bergmame et al. 2011; Sandland et al. 2014). Regardless, both species have been reported as having caused deaths in over 13 species of waterbird in areas where *B. tentaculata* occurs (Price 1934; Hoeve and Scott 1988; Bergmame et al. 2011; USGS NWHC, unpublished data). Unlike the closely related Greater Scaup (*A. marilis*), the distribution of Lesser Scaup is limited to the western hemisphere, and Lesser Scaup (hereafter, scaup) are thought to not yet have had sufficient time to develop co-evolutionary immunity to the introduced trematodes carried by the Faucet Snail, which is not reported to mediate die-offs within its native range (Herrmann and Sorensen 2009; Karatayev et al. 2012). The digeneans responsible for these mortality events are contracted once an infected intermediate host, the invasive Faucet Snail, is ingested, and death can result 3-10 days after the ingestion of a single snail (Hoeve and Scott 1988; Cole and Friend 1999). The importance of parasites as

population regulators is being re-examined due to these reoccurring scaup die-offs originating from the ingestion of infected *B. tentaculata*.

Several studies have investigated the parasite assemblages of scaup on their breeding and wintering grounds (Bush and Holmes 1986a,b; Vest 2002), but few have explored the helminth infracommunities (defined as the community of helminths occurring in a Lesser Scaup host) in spring-migrating scaup (Pillatzki 2007). Spring migration is an energetically expensive time in the annual cycle of waterfowl, especially for females that must not only replace endogenous resources expended during migration but also simultaneously develop reserves for subsequent breeding efforts. Therefore, it is important to understand the effects of parasitic infections on female scaup since future recruitment is tied to their condition and reproductive capacity (Anteau and Afton 2004). Intestinal helminths tend to reduce the condition of their host through direct and indirect means (e.g., reduced ability to forage, defend territories; lowered reproductive success; increased vulnerability to predators) (Møller 2005), and it is likely that the helminth assemblages of female scaup contribute to the Spring Condition Hypothesis more so than previously assumed.

Little is known about what helminths parasitize scaup during the spring, and even less is known about their impact on scaup health. The current dogma in ecology dismisses the idea that parasites have a substantial influence over host populations and tends to exclude them from food webs entirely (Tompkins et al. 2001; Hudson 2005, Lafferty et al. 2006). However, to ascertain the dynamic interactions associated with helminths and the declining scaup numbers, a description of all parasites infecting them during all periods of their annual cycle is needed to pave the way for future research into more complex host-parasite interactions (Pedersen and Fenton 2006). Spatiotemporal dynamics can influence how parasitic helminths limit and regulate scaup hosts through increased death rates and increased morbidity (Holt and Boulinier 2005). Furthermore, spatial and temporal heterogeneity can influence host-pathogen interactions by way of parasite infection and transmission, which may lead to reduced host condition (Holt and Boulinier 2005). A comprehensive examination of intestinal helminths in scaup is needed to

determine if they may be contributing to the Spring Condition Hypothesis. Here, I examined intestinal helminths of scaup to: 1) identify and differentiate helminthic intestinal infracommunities of female Lesser Scaup hosts in the Midwest during spring migration; 2) determine influence of host characteristics on infracommunity structure; and 3) compare these infracommunities across a spatiotemporal gradient within the study area. During the spring migrations of 2014 and 2015, I identified and enumerated intestinal helminths in 130 Lesser Scaup from stopover locations in 4 regions of the upper Midwest to examine the effects of host age and body size as well as the region and year of collection on helminth infracommunity structure (Santoro et al. 2012; Kanarek and Zaleśney 2013).

1.2 MATERIALS AND METHODS

Study Area

I collected female Lesser Scaup from wetlands and lakes within and nearby the Illinois and Mississippi River valleys and other important lakes and reservoirs in Illinois and western Wisconsin (Table 1.1, Figs. 1.1-2). In North America, the Lesser Scaup is the most abundant diving duck (USFWS, 2015) and constitutes approximately 89% of the continental scaup population (Lesser and Greater Scaup combined) (Austin et al. 2000). An estimated 75% of the continental scaup population travels through the Mississippi Flyway during spring and fall migrations. Regions of collection were categorized into 4 distinct areas post hoc based on latitude, physiography, and habitat type (Figs. 1.1-2): Southern Illinois (SI [Rend Lake]), the Illinois River Valley (IRV [Peoria Reach – Alton Reach]), the Central Mississippi River Valley (CMRV [Pools 12 and 19]), and the Upper Mississippi River Valley (UMRV [Pools 7 – 9]). I attempted to collect scaup in other areas of southern Illinois, eastern Illinois, and eastern Wisconsin, but samples from other areas were excluded due to relatively low numbers of scaup using these areas, insufficient sample sizes for analysis, or other reasons.

Collection and examination of Lesser Scaup

A total of 130 female Lesser Scaup were collected with shotguns from layout boats during spring migration (10 February 2014 – 20 April 2014 and 11 March 2015 – 12 April 2015). Flocks were first observed to confirm the foraging activity of individuals as part of a concurrent study examining scaup diets and wetland quality (Hagy et al. 2015). Due to the unpredictable and continuous movement of scaup during migration, collection sites were identified based on scaup usage during weekly ground and aerial surveys. After collecting a foraging female scaup, a global positioning system (GPS) waypoint was created at the collection site and identified with a unique alpha-numeric code. Immediately following collection and prior to necropsy, the lengths of the body (tip of the bill to distal retrix), culmen, wing chord, retrix, keel, and tarsometatarsus were measured to the nearest 1 mm for each individual (Afton and Ankney 1991; Carney 1992). Mass was recorded to the nearest 20 g using a spring scale both before and after necropsy. Two age classes, hatch year (HY) and after hatch year (AHY), were established based on retricies and wing plumage condition in conjunction with iris coloration (Trauger 1974; Carney 1992). Of the 130 female Lesser Scaup, 105 were determined to be AHY birds, 20 were HY, and 5 were unable to be aged due to damage during collection. However, the missing ages were estimated to be AHY based on 10,000 Expectation Maximization (EM) iterations using previously mentioned scaup morphometric measurements (Clarke et al. 2014).

Scaup were necropsied in situ within 10 to 15 minutes of collection. The body cavity was accessed via an incision along the keel. A gross examination of internal organs was conducted; and the intestinal tract (proximal duodenum to the cloacal vent) was sealed at each end with a zip-tie, excised from the body cavity, injected with and submerged in a marked plastic bag containing 70% ethanol (EtOH). The fixed intestines were refrigerated until helminth extraction could take place in the laboratory.

In the laboratory, the intestines were opened longitudinally from the cloaca to the proximal opening of the duodenum. Several species of intestinal helminth occur only in the ceca; thus, the ceca

were removed at the ileocecal junction and assessed for helminths separately under stereomicroscope. The separated intestinal and cecal lumena were each scraped and rinsed independently. The intestinal ingesta were repeatedly washed and concentrated in a conical beaker using a modified sedimentation technique (Pritchard 1982; Bush and Holmes 1986b). The washed ingesta were then transferred into a 250-mL graduated cylinder and brought to a total of 100 mL with fresh water. Using a magnetic stirrer and stir bar, the ingesta were thoroughly homogenized (all solids in the solution suspended), and a 10% subsample (10 mL) of the ingesta was removed using an aliquot technique (Bush and Holmes 1986b). The aliquot was transferred to a gridded 6 x 6 Petri dish and systematically examined under stereoscope. For each individual, recovered helminths were enumerated (total helminths = aliquot helminths x 10), and preserved using 70% EtOH. Nematodes and acanthocephalans were cleared in lactophenol for temporary mounts and identified to the lowest taxon using procedures found in Sepúlveda and Kinsella (2013). Following Pritchard (1982), trematodes and cestodes were stained using either Semichon's acetocarmine stain or Malzacher's staining technique, cleared in xylene, mounted in Canada balsam, and identified to the lowest taxon achievable (genus and/or species) using morphological characteristics under compound light microscopy and various helminth identification keys (McDonald 1974, 1981, 1988; Schmidt 1986; Kahlil et al. 1994). Helminths reported with the scientific epithet "sp" were not definitively classified at the species level, but were determined to be unique based on morphology and treated as such. Genera followed by the scientific epithet "spp" were typically immature individuals of already identified conspecifics whose underdevelopment rendered additional identification under our methods unsuccessful.

Data analysis

Following the definitions and statistical descriptions proposed by Bush et al. (1997), I examined the prevalence, abundance, intensity, total abundance, and species richness of parasite taxa of each bird (Santoro et al. 2012). Herein, I define 'prevalence' (P) as the percentage of scaup infected with a particular helminth taxa; 'intensity' (of infection) as the number of a particular helminth taxa in a single infected scaup; "abundance' as the number of individuals from a particular helminth taxa in an individual

host regardless of whether or not the host is infected; 'total abundance' (TA) as the number of all helminth taxa in each scaup; and 'species richness' as the number of helminth species recovered in each scaup. Mean values where calculated for abundance (MA; total number of individuals of a particular parasite species in a sample of scaup divided by total number of hosts) and intensity (MI; average intensity of a particular helminth taxa among infected members of host population). The 95% confidence intervals (CI) for prevalence were set using Sterne's exact method (Reiczigel 2003), and for mean values of intensity and abundance using the bias-corrected and accelerated bootstrap method with 20,000 replications (Ròzsa et al. 2000; Santoro et al. 2012). Because many of the species recovered from scaup specimens were congeneric and may exhibit similar effects on hosts, helminths were grouped into the taxa trematoda, cestoda, and nematoda, and mean values of each were calculated and compared among variables year and region as well as host age and size. Acanthocephalan helminths were excluded from further analysis because of their infrequent occurrence. Host size was indexed by conducting principal component analysis (PCA; PROC PRINCOMP) on scaup morphometrics (mass and body, culmen, wing chord, retrix, keel, and tarsometatarsus lengths) and using the first Principle Component (PC1) as an index of adjusted structural size (Afton and Ankney 1991; Vest 2002; Shutler et al. 2012). Brillouin's diversity index was calculated to determine infracommunity diversity (Santoro et al. 2012). The mean values of species richness, total abundance, and Brillouin's index of species diversity were used as infracommunity parameters for all helminths. Transformations ($\log_{10}$ [x + 1]) were used to normalize residuals of helminth infection parameters and separate analysis of variance (ANOVA; PROC GLM) was performed to assess the effects of year, region, age, and size and their interactions on infection parameters (Shutler et al. 2012). Non-significant predictor variables were sequentially removed from models by using partial sums of squares until only significant associations remained (Crawley 2005; Shutler et al. 2012). Statistically significant results ($\alpha = 0.05$) were investigated with post hoc pairwise comparisons between the terms in the factor region using Tukey's honest significant difference (HSD) testing (Zar 2010).

To compare helminth infracommunity structure, the helminth counts of all species were first 4^{th} root transformed to downweight the contributions of the highly abundant helminth species (Santoro et. al. 2012; Clarke et al. 2014). Then, a Bray-Curtis similarity coefficient was calculated for all scaup individuals and represented in a non-metric multidimensional scaling (NMDS) plot (Clarke et al. 2014). The number of NMDS dimensions was selected using 1,000 restarts for a maximum of 10 dimensions. A scree plot depicting each dimension and its corresponding stress statistic was assessed for an asymptotic plateau, thus indicating the appropriate number of NMDS dimensions to be used in subsequent analyses. Additionally, helminth infracommunity group means for each region and year term were obtained using 100 iterations of bootstrap averaging with 95% confidence ellipse and then plotted into NMDS ordination space to further assist in identifying spatiotemporal differences in infracommunity structure (Clarke et al. 2014, Clarke and Gorley 2015). The transformed Bray-Curtis index for helminth species was then correlated to each axis from the NMDS plot with Pearson correlation values. After sorting the Pearson correlation values from smallest to largest *r*-value for each axis, the top and bottom 5% ($n = 6$) of helminths were examined and determined to be driving the NMDS ordination of samples (Beals 2006).

To further investigate the effects of spatiotemporal and host parameters on helminth infracommunities, permutational multivariate analysis of covariance (PERMANCOVA) was implemented using the aforementioned Bray-Curtis similarity index (Anderson et al. 2008; Santoro et al. 2012). The PERMANCOVA procedure tests for the simultaneous response of one or more variables/co-variables in an analysis of covariance (ANCOVA) on the basis of a resemblance measure using permutation methods (Anderson et al. 2008). Region, year, and host age were treated as fixed effects, while the host size index was used as a covariate. Interaction terms were included for all main effects and the covariate, yet were sequentially removed from the final model due to a lack of statistical significance. The removal of the interaction terms increased analytical sensitivity and the proper interpretation of the model's main effects (Engqvist 2005; Santoro et al. 2012). The null hypothesis was constructed by 10,000 permutations and pseudo-F statistics were obtained (Anderson et al. 2008; Santoro et al. 2012). Type III sums of squares

were used to adhere to independence of all effects in the unbalanced model. Pairwise comparisons between the region variable's terms (IRV, CMRV, and UMRV) were further examined in PERMANCOVA. Due to small sample sizes and unbalanced collection of samples across years, the SI region was excluded from all statistical analyses with the exception of NMDS ordination. However, descriptive statistics for helminth and health parameter values are reported for the SI region.

The free software Quantitative Parasitology v. 3 (Reiczigel and Ròsza 2005) was used to create basic measures of helminth infection and their corresponding 95% CIs and execute comparisons (e.g., Chi-squared test and Fisher's exact test) of said infection measures. The statistical software package PRIMER v. 7 (Clarke and Gorley 2015) was utilized for performing the Expectation Maximization and NMDS ordination analyses. The addition PERMANOVA + for PRIMER v. 7 (Clarke et al. 2014) was used for PERMANCOVA procedures. All remaining analyses (e.g., summary statistics, ANOVA, Tukey's HSD) were done in SAS v. 9.4 (SAS Institute Inc., Cary, North Carolina, USA). Statistical significance for all analyses was set at $P < 0.05$.

1.3 RESULTS

Helminth identification and levels of infection

A total of 40 intestinal helminth taxa encompassing 20 trematodes, 14 cestodes, f nematodes, and 2 acanthocephala were identified in the parasitological examinations of 130 female Lesser Scaup collected from wetland areas of the upper Midwest, USA, during the spring migrations of 2014 and 2015 (Table 1.2). Out of the 647,174 helminths found, 44.6% were trematodes, 53.9% were cestodes, 1.4% were nematodes, and less than 0.05% were acanthocephalans (Table 1.3). All scaup collected were infected with 2–23 helminth taxa. All helminth species recovered were found to be of reproductively mature stages within scaup hosts, with the exception of an unidentified juvenile cestode (reported here as Juvenile). Infection parameters for each helminth species varied widely across the regional gradient (Table 1.2). Markedly prevalent helminth species regardless of region or year were the cestodes

Hymenolepis spinocirrosa and *H. pusilla*, which were present at a prevalence of ≥ 70% in each region (Table 1.2). The nematodes *Capillaria anatis*, *Baruscapillaria obsignata*, and *Strongyloides* sp. were also quite common in scaup from each region of the study (Table 1.2). General trends within the higher taxa helminth species were grouped into were less variable (Table 1.3). No differences among the spatiotemporal variables were detected between the mean helminth species richness or the abundances of the taxa cestoda and nematoda. Mean trematode abundance resulted in significant differences between years ($F_{1,118} = 13.8$, $p < 0.001$) with 2015 having fewer trematodes than 2014 ($t = 3.53$, $p < 0.001$; Fig. 1.3). Additionally, the digeneans assumed responsible for the annual scaup die-offs, *C. bushiensis* and *Sphaeridiotrema* spp., were limited in their distributions to Pool 12 of the CMRV and the entirety of the UMRV (Table 1.2). Infections of *C. bushiensis* and *Sphaeridiotrema* spp. did not differ between 2014 and 2015 ($p = 0.09$ and $p = 0.34$ respectively). One digenean, *Plenosoma minimum*, is reported for the first time in scaup as well as for occurring in the upper Midwest with prevalence ranging from 1.7% to 52.6%.

Host age and size were not associated with any aspects of infracommunity structure; however, several metrics differed by year and region (Table 1.4). Mean total helminth abundance differed between years ($F_{1,118} = 7.9$, $p = 0.01$) (Fig. 1.4), with 2014 comprising 68.3% (2014; 6,997.2/10,245 vs. 2015; 3,247.8/10,245) of the mean total abundance. The Brillouin's species diversity index differed by region ($F_{2,117} = 5.5$, $p = 0.005$) (Table 1.4) with the UMRV having greater diversity than the CMRV ($t = 3.25$, $p = 0.004$) (Fig. 1.5).

Helminth infracommunity comparisons

The 4D ordination displayed the most significant decrease in stress and was chosen as the representative dimension for the original data. The 2D ordination plot resulted in a relatively high stress (0.28), whereas the 4D ordination resulted in a lower stress level (0.17) for the helminth infracommunity comparisons across regions and years (Figs. 1.6-7). Bootstrap averages plotted in 2D resulted in well defined, distinguishable infracommunity groups based on year of collection within the 4D ordination (Fig.

1.8). The bootstrap averages between all infracommunity comparisons and means of comparisons by region were also well separated with the exception of the overlapping 95% confidence ellipses of UMRC and CMRV in the 2D representation of the first two axes (Fig. 1.8). Regional separation was most evident in the 3D NMDS plot demonstrating structural helminth infracommunity differences across the study area (Fig. 1.9). The Pearson correlations of the Bray-Curtis similarity index infracommunities and each of the 4D NMDS axes revealed the helminth species influencing these ordination patterns (Table 1.5). *Microphallus oblonga* had the strongest negative correlation (r = -0.55) with axis 1, and *Echinoparyphium recurvatum* displayed the strongest positive correlation (r = 0.75). Axis 2 was highly influenced by the negative loadings of the cestodes *H. pusilla* (r = -0.73) and *H. spinocirrosa* (r = -0.72), which were found to be prevalent in > 75% of collected scaup (Table 1.2). *Microphallus oblonga* was most prevalent in 2015 (P = 31.4%; p = 0.021) in the northerly regions of the study area (CMRV [P = 36.4%, MI = 1951.5]; UMRV [P = 28.9%, MI = 85.8]). The mean infection of *E. recurvatum* was greater in the IRV (P = 85.0%, MI = 1831.3) than other regions and proportions of infected host scaup were greater in 2014 than in 2015 ($p < 0.001$) (Table 1.2).

The PERMANCOVA results indicated that region (Pseudo-$F_{2,114}$ = 5.5, $p < 0.001$) and year (Pseudo-$F_{1,114}$ = 6.0, $p < 0.001$) had a significant impact in the structure of the helminth infracommunities. Pairwise comparisons between the region terms revealed that infracommunity structures were different amongst all region of the study area (Table 1.7). With regard to scaup intrinsic variables, neither age (Pseudo-$F_{1,114}$ = 0.9, p = 0.55), nor the body size covariate (Pseudo-$F_{1,114}$ = 1.7, p = 0.052) were significant predictor variables of helminth infracommunity structure.

1.4 DISCUSSION

The structure of helminth infracommunities in scaup migrating through the upper Midwest in spring was spatiotemporally variable, in that helminth assemblages changed with latitude and time. Such fluctuations in infracommunity structure are important because different helminth assemblages and

varying rates of species turnover may exhibit different energetic consequences on spring migrating scaup. Global climate change and increased habitat fragmentation are of increasing concern for conservation efforts (Austin et al. 2006; Drever et al. 2012) and may affect the dispersal of hosts and parasites thereby altering their interactions and disease dynamics at different scales (Holt and Boulinier 2005). Considering the temporal proximity of the spring migration and breeding seasons, energetic and behavioral costs of immune responses to varying helminth infections may lead to reduced survival and/or productivity (Hanssen et al. 2004; Stafford et al. 2014). The differing helminth infracommunity structures observed herein occurred over a relatively small geographical scale indicating that scaup may experience frequent and rapid changes in intestinal parasite communities, and this change is likely related to intermediate hosts present in scaup diets.

Differences between years were likely due to climatic factors influencing timing and duration of scaup migration chronology and emergence and viability of intermediate hosts and the parasites they harbor. In a study of Blue-Winged Teal (*Anas discors*), Wallace and Pence (1986) found that the helminths of Blue-Winged Teal may have been acquired in areas farther south of their study area either prior to or earlier in migration (e.g., Gulf Coast wintering grounds, stopover sites). This scenario may apply to scaup as well, yet scaup display one of the most protracted spring migrations of any North American waterfowl species (Bellrose 1976). Moreover, scaup may remain at a stopover location in the upper Midwest > 10 days, which may be longer than other species (Krementz et al. 2011; Hagy et al. 2015). Generally, intestinal helminths are relatively short lived in scaup (1-2 weeks), and their abundance and infracommunity structure in a scaup host depends on their ability to persist in the environment (Gagnon 1990; Huffman 2008). Turnover of helminth infracommunities often occurs from shifts in diet during migration across the landscape and can be either chemically induced or from the abrasive scrapping of the intestinal walls from mollusk shells (McLaughlin and Burt 1973). Therefore, scaup continuously shed and replace helminths from their immediate environment, thus leading to an ongoing shift in intestinal helminth infracommunities during migration. Though the impacts of continuous shifts in

helminth infracommunity structure are not well understood, parasites have evolved to exploit host resources and to influence infection and/or transmissibility, and my data shows that parasite infracommunities can change rapidly and are likely linked to regional variation in habitat and prey base (Brown et al. 2005).

The differences in helminth infracommunity structure between years, may have been due to a climate impact on the food base (intermediate hosts) of scaup. The winter of 2014 was long and cold compared to the mild winter of 2015. In central Illinois, USA, the average temperature during the winter of 2014 (1 December 2013 – 28 February 2014) was -4.2 ºC lower than the long term average of -2.4 ºC, and degree days below 0 ºC were 12.8 days greater the normal 7.2 (NOAA 2015). Furthermore, snow accumulation during 2014 totaled 126.0 cm (NOAA 2015), a 74.7 cm increase over the long term average total of 51.3 cm. By comparison, winter 2015 only experienced a slight deviation of -0.9 ºC from the long term mean temperature, and 8 degree days below 0 ºC (NOAA 2015). Snow accumulations totals for the period were slightly above normal at 58.4 cm, and the winter season was interrupted by a brief, uncharacteristic warming period in early spring (NOAA 2015). Although climactic variations were not specifically recorded as a part of this study, such abiotic factors undoubtedly play an integral part in the life strategies of many of the helminths observed here (Lafferty and Kuris 2005; Poulin and Mouritsen 2006; Mas-Coma et al. 2009).

Study regions differed on the basis of latitude, geomorphology, hydrologic patterns, habitat structure, and forage type and availability. The most southerly region (SI) was represented by a man-made reservoir, Rend Lake, which is deep and experiences comparatively stable water levels by way of dam control. Spring migration stopovers at Rend Lake usually coincided with the breakup of ice on the reservoir, and scaup primarily consumed American Gizzard Shad (*Dorosoma cepedianum*), an atypical food item (Anteau and Afton 2008a; Hagy et al. 2015). Although some fish species are capable of transmitting helminths to waterbirds (Kanarek and Zaleśney 2013), helminths found during this project are typically transmitted by a scaup's more preferred prey items (i.e., amphipods, gastropods, bivalves)

(McDonald 1974, 1981, 1988). The small sample size of scaup in concert with the limited diversity of prey items and the anthropogenic influence over water levels may be determinant factors reducing helminth species richness, diversity, and abundance in this region.

The IRV differs from the other regions of the study area in that it is a riverine system regulated by a series of locks and dams with a diversity of primary habitat used by scaup, including nutrient-rich connected backwater lakes devoid of aquatic vegetation due to dynamic water levels, partially connected backwater lakes and wetland managed for moist-soil vegetation production, and disconnected flood-plain lakes with abundant submersed, emergent, and floating-leaf aquatic vegetation. Possibly related to this habitat diversity, the IRV had a higher diversity of invertebrate food items for foraging scaup than SI, CMRV, and UMRV (Hagy et al. 2015), and only lacked four, of the 40 helminth taxa reported herein. Two species (*C. bushiensis*, and *Sphaeridiotrema* spp.) were only found in the UMRV and Pool 12 of the CMRV. Although not specifically examined, I speculate that intermediate host diversity is most likely the cause of higher helminth diversity in scaup. Unlike SI where scaup foraged almost exclusively on dead American Gizzard Shad, the regions sampled within the Illinois and Mississippi River complexes contained a diverse suite of animal and plant food items (Hagy et al. 2015). In these regions, invertebrate food items consumed by scaup (n = 262) occurred in diets at a frequency of 82% with the taxa chironomidae (37%), amphipoda (30%), physiidae (29%), sphaeriidae (27%), and Zebra Mussels (21%) occurring most frequently (Hagy et al. 2015). Of the 17 invertebrate prey items reported by Hagy et al. (2015), 6 belonged to gastropoda taxa and 3 to bivalvia. Many of the species eaten by scaup are often utilized as intermediate hosts for helminths infecting scaup and other waterbirds (Spasskaya 1966; McDonald 1981; Kahlil et al. 1994; Huffman 2008).

Like the IRV, the CMRV is more dynamic and species rich in a scaup forage than SI, most likely due to the fact that it is part of a large riverine system with diverse communities of submersed and floating leaf aquatic vegetation and large shallow flats where foraging is common. Pool 19 is a 12,329.2 ha impounded section of the Mississippi River behind a lock and dam, which stabilized water levels and

allows growth of aquatic vegetation. Pool 19 has abundant mussel and invertebrate populations and has been a major stopover and waterfowl research location for scaup for many years (Bellrose 1974; Anteau and Afton 2004, 2006; Baldassarre 2014; Hagy et al. 2015). In contrast, Pool 12 is located nearly 221 km north of Pool 19 and is not as rich in forage items as Pool 19 (Hagy et al. 2015). Furthermore, Pool 12 lies near the southern terminus of the Faucet Snail's range in the Mississippi River and may be influencing the helminth infracommunity similarity indices due to the presence of the non-native trematodes there.

The UMRV encompassed Mississippi River Pools 7 through 9, which are the pools where biannual scaup die-offs have occurred since the early 2000s (Sauer et al. 2007; Herrmann and Sorensen 2009, 2011). Helminth infracommunity differences between this region and the other regions in this study were most evident because of the elevated prevalence of *C. bushiensis*, *Sphaeridiotrema* spp., and *P. minimum*. Although, spatiotemporal comparisons for the UMRV in 4D NMDS ordination space slightly overlapped with the CMRV, mean trematoda abundance, total helminth abundance, and Brillouin's species diversity index were highest in this region. The UMRV is similar to Pool 12 of the CMRV in scaup forage availability with the exception of *B. tentaculata*, which was more abundant in the pools of the UMRV (Sauer et al. 2007). Artificial islands created in the back waters of Pool 7 provide exceptional breeding areas for *B. tentaculata* because of the shallow waters and riprap used in island construction (Herrmann and Sorensen 2009, 2011). During collection in this region, scaup were frequently seen diving near the islands where the Faucet Snail has been previously reported occurring in high densities. One can only presume that scaup were foraging on *B. tentaculata*, since the concurrent scaup diet study did not report taxonomic resolution to species for gastropods (Hagy et al. 2015). The increased prevalence of *C. bushiensis* and *Sphaeridiotrema* spp. may be explained based on Faucet Snail abundance and scaup foraging habits in the UMRV.

With the exception of nematodes, the helminth taxa reported herein display indirect life cycles that typically require an obligate intermediate host (usually a gastropod), followed by a second intermediate host (gastropods, bivalves, amphibians, fishes), and finally an avian definitive host (Huffman

2008). Scaup serve as a definitive host for all helminth taxa reported here with the possible exception of the recovered unidentified juvenile cestode and *Echinostoma trivolvis*, of which, no sexually mature adults were found for either helminths. Of the 20 trematodes encountered, 18 have been identified as causing disease, epizootic events, and/or death in North American hosts of the order Anseriformes (McDonald 1981 and references herein; Huffman 2008). Species of the genera *Apatemon*, *Cotylurus*, *Echinoparyphium*, *Echinostoma*, *Psilochasmus*, *Maritrema*, *Microphallus*, *Notocotylus*, *Paramonostomum*, and *Zygocotyle* are capable of negatively affecting scaup condition and were found parasitizing scaup in this study (McDonald 1981). *Cyathocotyle bushiensis* and *Sphaeridiotrema* spp. were not found outside of the currently known distribution of the Faucet Snail (Sauer et al. 2007). *Cyathocotyle bushiensis* was only found to occur in the ceca of scaup, but *Sphaeridiotrema* spp. were found infecting both intestines and ceca, though generally amassing within the former. Differentiation between the introduced *Sphaeridiotrema* species (*S. globulus* and *S. pseudoglobulus*) known to infect waterbirds in the upper Midwest was not possible in this study, although research by Bergmame et al. (2011) on Pool 7 of the Mississippi River identified all *Sphaeridiotrema* species collected during their research as *S. pseudoglobulus* based on DNA analyses. Regardless, both species cause mortality in many North American waterbirds and have similar life cycles.

In North America, *P. minimum* has previously only been reported in Black Oystercatchers (*Haematopus bachmani*) and South Pacific Sea Otters (*Enhydra lutris nereis*), and is known to cause pathology in the latter host (Helminthological Society of Washington 1964; Mayer et al. 2003). Oddly, *P. minimum* appeared in all regions of the current study except for SI, yet approximately 97% of scaup harboring *P. minimum* were collected in the UMRV. An explanation of the high prevalence of *P. minimum* in the region was not evident in this study, and a literature review revealed no references of *P. minimum* having ever occurred in the Midwest or scaup hosts. There may be two possibilities for this occurrence: 1) *P. minimum* has been introduced into these areas during migration events and is now persistent 2) the *P. minimum* specimens recovered were parasitizing scaup migrating from the southern

California coast where the helminth is known to occur. Considering relatively few scaup winter in the southern portion of the Pacific Flyway (~16%; Baldassarre 2014) and fewer still migrate between flyways, the second scenario seems highly unlikely. It is more plausible that *P. minimum* has been spread to the Midwest by other means (e.g., waterbird migrations, invasive species introductions) and has become established due to favorable environmental conditions and suitable intermediate freshwater hosts. The remaining trematodes found in scaup have been reported parasitizing scaup or closely related congenerics (McDonald 1981; Bush and Holmes 1986a,b; Vest 2002).

Cestodes comprised 14 individual taxa and nine species that occurred throughout all regions and years of the study area. Like trematodes, the majority of cestodes are hermaphroditic and exhibit an indirect life cycle involving several intermediate hosts before reaching sexual maturity in a definitive host. The hymenolepids *Dicranotaenia coronula, Fimbriaria fasciolaris, Sobolevicanthus* sp., and *H. megalops* have been reported to cause pathology, disease, and mortality within Anseriformes hosts (Wobeser 1974; Basu et al. 1982). In scaup, infections with cestodes may indirectly affect population size through reduced host fitness and reproductive success (McLaughlin 2008). All identified taxa herein have been reported parasitizing scaup and their relatives (Spasskaya 1966; Schmidt 1986; Kahlil et al. 1994).

Only four nematode species from two genera were observed parasitizing scaup, and most individuals were found within the ceca. The capillarids *C. anatis* and *B. obsignata* are considered to be characteristically common helminths infecting waterfowl, including scaup, and were found in each region of the study during both years (McDonald 1981). In contrast, *C. spinulosa* occurred less frequently and was only found in scaup from the IRV. Unlike the trematodes and cestodes discussed previously, these capillarids exhibit a direct life cycle as helminth eggs are released into the environment through host excrement, embryonate, and are then incidentally ingested by foraging waterfowl (Yabsley 2008). *Capillaria* species often occur at high prevalences but low intensities and rarely cause disease in wild hosts (Yabsley 2008). *Strongyloides* species occurred in all regions and years of the study and have been reported in scaup breeding in Alberta, Canada (Bush and Holmes 1986b). Surprisingly, the literature

containing life history characteristics about the *Strongyloides* species infecting waterfowl is scant. However, McDonald (1974) reported strongyloid infections being entirely composed of females since the species alternates from free living to parasitic generations and only female individuals may become parasitic. In hosts outside of the order Aves, *Strongyloides* species exhibit a high degree of host specificity, and they typically infect hosts by migrating into and through the skin (Viney and Lok 2007). Similar to capillarids, strongyloids rarely produce overt signs of pathology unless host health is compromised and unable to initiate an immune response (Viney and Lok 2007).

Acanthocephalans were the least frequently encountered intestinal parasites of scaup. Only *Polymorphus marilis* and *Corynosoma constrictum* were found, and like most species reported herein, have been observed in previous studies (Bush and Holmes 1986a,b; Vest 2002). Acanthocephalans require at least one intermediate host; amphipods, isopods, and decapods are common vectors of the parasites in waterfowl (Cole and Friend 1999). Bethel and Holmes (1977) described how acanthocephalan larvae (cystacanths) are capable of altering the behavior and sometimes coloration of their amphipod host in order to increase amphipod predation by waterbirds and fish thus perpetuating the helminth's lifecycle. Several acanthocephalan species have been documented as the etiological agent causing decreased health (e.g., reduced weight gain and altered blood metabolite ratios), lowered reproduction, and die-offs of various sea duck species (e.g., Common Eiders [*Somateria mollissima*]) in arctic regions of North America and Europe (Hollmén et al. 1999; Skerratt et al. 2005).

Helminth infracommunity structure of scaup migrating through the region was spatiotemporally variable, with helminth assemblage changing according to region and year. Observed differences in helminth species prevalence, abundance, and diversity were presumably driven by the timing of migration, annual climatic variation, and regional prey diversity. Hechinger and Lafferty (2005) documented correlations between avian host species richness, species heterogeneity, and abundance, and the species richness, species heterogeneity, and abundance of trematodes from intermediate snail hosts. Considering the diversity of invertebrate species utilized by scaup as food items in the study area (Hagy et

al. 2015), and the fact that over 40% of continental populations of nearly 30 different North American waterfowl species use the Mississippi Flyway for seasonal migrations (Wasburn 2008; Hagy et al. 2015) (and therefore regions examined in this study), it is reasonable to expect that parasite diversity would be high in the regions of my study area during periods of scaup migration. Future research objectives should be placed on identifying the effects of these helminth infections on scaup hosts as well as unraveling the complex, underlying mechanisms associated with host-parasite interactions as well as their effects on body condition of migrating waterfowl. Increasing our understanding of the abiotic and biotic factors influencing helminth infections and identifying their impacts on scaup health and condition, will assist in future conservation efforts for this species of concern.

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1.6 TABLES AND FIGURES

Table 1.1. Number of female Lesser Scaup (*Aythya affinis*) collected from 4 regions of the upper Midwest, USA, during the spring 2014 and 2015, including the Upper Mississippi River Valley (UMRV), Central Mississippi River Valley (CMRV), Illinois River Valley (IRV), and Southern Illinois (SI).

Region	Dates of Collection	
	10 February - 20 April 2014	11 March - 12 April 2015
	n	
UMRV	12	26
CMRV	10	12
IRV	38	22
SI*	0	10
Total	60	70

*Lesser Scaup were not collected from SI during 2014.

Table 1.2. Infection parameters of the intestinal helminth species of 130 female Lesser Scaup (*Aythya affinis*) collected from 4 regions of the upper Midwest, USA, during spring 2014 and 2015, including the Upper Mississippi River Valley (UMRV), Central Mississippi River Valley (CMRV), Illinois River Valley (IRV), and Southern Illinois (SI). P: prevalence (% infected scaup). MI: mean intensity (mean number of helminths from infected scaup). Numbers in parentheses are the 95% confidence intervals for parameters.

Taxon	SI (n = 10)		IRV (n = 60)		CMRV (n = 22)		UMRV (n = 38)	
	P	MI	P	MI	P	MI	P	MI
Trematoda								
Apatemon gracilis	10.0 (0.5-44.6)	10.0	20.0 (11.4-32.4)	32.4 (21.3-44.4)	22.7 (9.4-45.3)	221.2 (32.6-457.8)	39.5 (21.4-60.9)	475.9 (88.3-914.3)
Apatemon burti	-	-	5.0 (1.4-13.9)	7.0 (1.0-10.0)	4.5 (0.2-22.2)	2.0	2.6 (0.1-14.0)	60.0
Cotylurus cornutus	-	-	4.7 (0.1-8.9)	10.0	-	-	5.3 (1.0-18.0)	15.0 (10.0-15.0)
Cotylurus flabelliformis	10.0 (0.5-44.6)	20.0	6.7 (2.3-16.4)	52.5 (20.0-65.0)	9.1 (1.6-29.1)	174.5 (97.0-174.5)	7.9 (2.2-20.8)	79.3 (40.0-114.3)
Cotylurus gallinulae hebraicus	10.0 (0.5-44.6)	20.0	25.0 (15.6-37.4)	45.9 (25.9-82.9)	18.2 (6.5-38.9)	43.0 (11.5-102.5)	34.2 (20.8-51.3)	67.2 (41.2-99.1)
Cotylurus spp.*	10.0 (0.5-44.6)	10.0	31.7 (20.6-45.0)	33.2 (22.6-55.0)	22.7 (9.4-45.3)	139.2 (30.0-336.0)	39.5 (24.7-55.7)	159.3 (39.9-620.6)
Cyathocotyle bushiensis	-	-	-	-	-	-	36.8 (22.6-53.5)	27.7 (10.0-92.0)
Diplostomum phoxini	-	-	13.3 (6.3-24.8)	44.4 (10.6-96.9)	9.1 (1.6-29.1)	20.0 (10.0-20.0)	2.6 (0.1-14.0)	30.0
Echinoparyphium recurvatum	20.0 (3.7-55.4)	409.0 (10.0-409.0)	85.0 (73.5-92.2)	1831.3 (1200.3-3540.5)	31.8 (15.2-54.7)	57.1 (17.1-170.0)	52.6 (36.7-68.7)	262.6 (160.0-402.2)
Echinostoma trivolvis	20.0 (3.7-55.4)	32.5 (30.0-32.5)	36.7 (24.8-50.0)	436.0 (122.4-1557.4)	13.6 (3.8-33.8)	9.3 (1.0-14.0)	36.8 (22.6-53.5)	71.4 (33.1-181.9)
Psilochasmus oxyurus	10.0 (0.5-44.6)	31.0	40.0 (28.2-53.4)	33.9 (21.5-58.5)	40.9 (22.2-61.7)	35.0 (18.0-80.1)	23.7 (12.7-39.4)	53.7 (21.3-110.6)
Maritrema sp. 1	-	-	3.3 (0.6-11.4)	296.0 (174.0-296.0)	4.5 (0.2-22.2)	20.0	13.2 (5.3-27.4)	172.4 (50.0-434.0)
Maritrema sp. 2	10.0 (0.5-44.6)	10.0	-	-	18.2 (6.5-38.9)	14.6 (4.3-25.0)	-	-
Microphallus oblonga	-	-	18.3 (10.1-29.9)	121.4 (60.9-190.2)	36.4 (18.7-58.2)	1951.5 (827.1-3937.5)	28.9 (16.7-45.3)	85.8 (49.8-136.3)

Table 1.2. Continued

Taxon	SI (*n* = 10)		IRV (*n* = 60)		CMRV (*n* = 22)		UMRV (*n* = 38)	
	P	MI	P	MI	P	MI	P	MI
Microphallus pygmaeus	-	-	-	-	27.3 (12.6-50.0)	9916.0 (2946.7-16885.3)	7.9 (2.2-20.8)	1433.7 (161.0-2576.7)
Microphallus spp.*	-	-	11.7 (5.6-22.3)	147.7 (29.6-340.1)	18.2 (6.5-38.9)	3617.5 (235.0-9121.5)	15.8 (7.1-31.3)	1624.2 (404.7-4029.7)
Notocotylus sp.	30.0 (8.7-61.9)	3.0 (2.0-4.0)	6.7 (2.3-16.4)	10.8 (1.0-20.5)	13.6 (3.8-33.8)	14.7 (1.0-28.3)	2.6 (0.1-14.0)	1.0
Paramonostomum sp.	-	-	5.0 (1.4-13.9)	8.7 (7.0-10.0)	4.5 (0.2-22.2)	1.0	-	-
Plenosoma minimum	-	-	1.7 (0.1-8.9)	10.0	9.1 (1.6-29.1)	55.5 (1.0-55.5)	52.6 (36.7-68.7)	1816.2 (943.2-3100.1)
Prosthogonimus anatinus	-	-	3.3 (0.6-11.4)	6.0 (1.0-6.0)	-	-	2.6 (0.1-14.0)	10.0
Sphaeridiotrema spp. ł	-	-	-	-	54.5 (33.8-74.0)	258.4 (107.5-577.6)	71.1 (54.7-93.3)	454.3 (236.7-909.5)
Zygocotyle lunata	20.0 (3.7-55.4)	1.0	41.7 (29.9-55.0)	9.4 (5.0-24.4)	31.8 (15.2-54.7)	8.6 (2.3-24.9)	18.4 (8.8-34.0)	2.3 (1.4-3.1)
Cestoda								
Anatinella sp.	-	-	1.7 (0.1-8.9)	10.0	4.5 (0.2-22.2)	170.0	-	-
Dicranotaenia coronula	50.0 (22.3-77.8)	6.0 (2.6-10.8)	40.0 (28.2-53.4)	103.3 (51.3-210.3)	45.5 (21.1-66.2)	9.9 (5.2-17.4)	39.5 (24.7-55.7)	42.1 (17.7-122.5)
Diorchis sp. 1	30.0 (8.7-61.9)	4.3 (1.0-7.3)	40.0 (28.2-53.4)	113.2 (58.9-289.5)	40.9 (22.2-61.7)	514.1 (93.1-1730.9)	28.9 (16.7-45.3)	118.8 (40.6-355.7)
Diorchis sp. 2	10.0 (0.5-44.4)	10.0	28.3 (18.2-40.8)	47.6 (25.1-101.1)	40.9 (22.2-61.7)	157.8 (17.3-702.2)	28.9 (16.7-45.3)	104.9 (26.2-383.4)
Echinocotyle rosseteri	-	-	20.0 (11.4-32.4)	413.9 (185.3-796.3)	9.1 (1.6-29.1)	75.0 (65.0-75.0)	15.8 (7.1-32.3)	1539.5 (81.7-5352.8)
Fimbriaria fasciolaris	10.0 (0.5-44.6)	10.0	45.0 (32.4-57.8)	845.1 (231.2-3155.1)	45.5 (26.1-66.2)	73.4 (31.8-135.9)	28.9 (16.7-45.3)	33.9 (17.6-59.7)
Fimbriariodes intermedia	-	-	5.0 (1.4-13.9)	34.3 (1.0-67.3)	18.2 (6.5-38.9)	42.5 (5.5-93.8)	7.9 (2.2-20.8)	36.3 (21.0-48.3)
Hymenolepis megalops	-	-	5.0 (1.4-13.9)	195.0 (2.0-385.3)	4.5 (0.2-22.2)	10.0	2.6 (0.1-14.0)	1.0

Table 1.2. Continued

Taxon	SI (*n* = 10)		IRV (*n* = 60)		CMRV (*n* = 22)		UMRV (*n* = 38)	
	P	MI	P	MI	P	MI	P	MI
Hymenolepis pusilla	90.0 (55.4-99.5)	309.9 (140.8-757.4)	78.3 (65.9-87.3)	1343.6 (740.0-2446.8)	72.7 (50.0-87.4)	1467.8 (587.3-3326.4)	71.1 (54.7-83.3)	1618.9 (838.2-3586.9)
Hymenolepis spinocirrosa	70.0 (38.1-91.3)	576.7 (229.7-1064.0)	91.7 (81.9-96.7)	1449.1 (897.4-2430.5)	77.3 (54.7-90.6)	1211.2 (589.1-2175.6)	71.1 (54.7-83.3)	829.7 (396.9-1771.4)
Hymenolepis tuvensis	20.0 (3.7-55.4)	102.0 (100.0-102.0)	23.3 (13.9-35.7)	630.0 (209.9-1552.9)	22.7 (9.4-45.3)	556.2 (34.2-1982.4)	31.6 (18.0-48.7)	89.8 (48.8-202.7)
Hymenolepis spp. *	-	-	33.3 (22.3-46.6)	248.9 (126.3-549.5)	27.3 (12.6-50.0)	232.5 (53.2-536.2)	34.2 (20.8-51.3)	113.9 (65.6-217.1)
Retinometra macrocanthos	20.0 (3.7-55.4)	20.0	40.0 (28.2-53.4)	301.6 (155.6-640.8)	36.4 (18.7-58.2)	136.8 (45.9-281.4)	21.1 (10.0-36.7)	137.1 (55.3-314.1)
Sobolevicanthus sp.	10.0 (0.5-44.6)	7.0	23.3 (13.9-35.7)	150.4 (42.8-548.0)	18.2 (6.5-38.9)	163.3 (8.3-465.0)	26.3 (14.0-42.1)	25.0 (8.2-53.0)
Juvenile†	-	-	3.3 (0.6-11.4)	495.5 (10.0-495.5)	-	-	-	-
Nematoda								
Baruscapillaria obsignata	60.0 (29.1-85.0)	7.7 (4.0-12.8)	30.0 (19.6-42.9)	15.0 (10.2-20.9)	59.1 (38.3-77.8)	6.8 (4.9-9.6)	47.4 (31.4-63.3)	8.6 (6.4-12.7)
Capillaria anatis	50.0 (22.3-77.8)	4.8 (2.4-7.2)	66.7 (53.4-77.7)	15.2 (11.8-21.1)	40.9 (22.2-61.7)	13.9 (7.8-20.4)	57.9 (42.0-72.6)	11.1 (7.5-17.5)
Capillaria spinulosa	-	-	3.3 (0.6-11.4)	3.0 (1.0-3.0)	-	-	-	-
Capillaria spp.*	10.0 (0.5-44.6)	2.0	25.0 (15.6-37.4)	3.9 (2.5-6.5)	22.7 (9.4-45.3)	5.2 (1.2-12.8)	34.2 (20.8-51.3)	9.4 (6.0-18.6)
Strongyloides sp.	60.0 (29.1-85.0)	19.0 (5.7-45.0)	30.0 (19.6-42.9)	42.3 (21.4-88.6)	31.8 (15.2-54.7)	62.7 (27.1-103.9)	55.3 (39.4-70.4)	294.6 (16.2-1383.0)
Acanthocephala								
Corynosoma constrictum	-	-	6.7 (2.3-16.4)	21.5 (5.5-37.8)	4.5 (0.2-22.2)	11.0	-	-
Polymorphus marilis	10.0 (0.5-44.6)	2.0	8.3 (3.4-18.1)	8.8 (3.2-14.6)	9.1 (1.6-29.1)	59.0 (2.0-59.0)	-	-

*****Species were unable to be identified to be differentiated between previously identified congeneric species. ǂ *Sphaeridiotrema* specimens could not be differentiated between *S. globulus* and *S. pseudoglobulus*. † Juvenile refers to a cestode specimen where all individuals were underdeveloped and unidentifiable.

Table 1.3. Mean abundance values (95% confidence intervals in parentheses) of intestinal helminth taxa found in 130 female Lesser Scaup (*Aythya affinis*) collected from 4 regions of the upper Midwest, USA, during spring 2014 and 2015, including the Upper Mississippi River Valley (UMRV), Central Mississippi River Valley (CMRV), Illinois River Valley (IRV), and Southern Illinois (SI). Scaup age terms include: Hatch Year (HY) and After Hatch Year (AHY).

	n	Trematoda	Cestoda	Nematoda	Acanthocephala
Region					
UMRV	38	2167.1 (1032.4-3301.7)	2179.8 (665.5-3694.0)	176.5 (0-478.1)	-
CMRV	22	4367.4 (272.0-8462.7)	2608.1 (913.0-4303.2)	30.9 (11.4-50.4)	5.9 (-6.1-17.9)
IRV	60	1823.1 (923.4-2722.9)	3357.9 (1876.6-4839.2)	28.4 (17.0-39.8)	2.2 (0.1-4.2)
SI	10	99.5 (-99.9-298.9)	714.0 (235.6-1192.4)	18.6 (3.8-33.4)	0.2 (0-0.7)
Year					
2014*	60	3565.0 (1837.1-5292.9)	3401.3 (1810.5-4992.0)	29.8 (14.5-45.1)	1.2 (0-2.4)
2015	70	1070.2 (603.7-1536.8)	2067.8 (1247.1-2888.5)	107.0 (0-268.1)	2.7 (0-6.6)
Age					
HY	20	2187.6 (598.5-3776.7)	2261.2 (737.7-3784.6)	28.6 (10.3-46.9)	0.8 (0-1.9)
AHY	110	2227.8 (1255.5-3200.2)	2760.0 (1784.2-3735.8)	79.1 (0-181.3)	2.2 (0-4.8)

*Lesser Scaup were not collected from SI during 2014.

Table 1.4. Mean values (95% confidence intervals in parentheses) of infracommunity parameters of intestinal helminths found in 130 female Lesser Scaup (*Aythya affinis*) collected from 4 regions of the upper Midwest, USA, during spring 2014 and 2015, including the Upper Mississippi River Valley (UMRV), Central Mississippi River Valley (CMRV), Illinois River Valley (IRV), and Southern Illinois (SI). Scaup age terms include: Hatch Year (HY) and After Hatch Year (AHY). Total abundance refers to all helminth taxa found.

	n	Total Abundance	Species Richness	Brillouin's Index
Region				
UMRV	38	4523.4 (2144.6-6902.1)	11.0 (9.8-12.1)	1.343 (1.178-1.507)
CMRV	22	7012.2 (2636.7-11387.7)	10.2 (8.0-12.4)	0.926 (0.685-1.167)
IRV	60	5211.6 (3419.6-7003.6)	10.1 (9.2-10.9)	1.128 (1.015-1.242)
SI	10	832.3 (353.7-1310.9)	6.5 (4.7-8.3)	0.809 (0.533-1.085)
Year				
2014*	60	6997.2 (4564.2-9430.1)	10.1 (9.1-11.0)	1.181 (1.060-1.301)
2015	70	3247.8 (2168.8-4326.1)	10.1 (9.1-11.1)	1.090 (0.966-1.214)
Age				
HY	20	4478.1 (1745.1-7211.1)	10.4 (8.0-12.7)	1.105 (0.851-1.359)
AHY	110	5069.2 (3615.9-6522.5)	10.0 (9.4-10.7)	1.137 (1.044-1.230)

*Lesser Scaup were not collected from SI during 2014.

Table 1.5. Helminths determined to be most influential on non-metric multidimensional scaling (NMDS) ordination plot formation. Values represent the Pearson correlations (*r*) obtained between each NMDS axis and the 4th root transformed Bray-Curtis similarity index of all intestinal helminths from 130 female Lesser Scaup (*Aythya affinis*) collected from 4 regions of the upper Midwest, USA, during spring 2014 and 2015.

Axis	Taxon Pearson Correlation Value					
	Microphallus oblonga	*Sphaeridiotrema* spp.	*Strongyloides* sp.	*Hymenolepis spinocirrosa*	*Zygocotyle lunata*	*Echinoparyphium recurvatum*
NMDS1	-0.55	-0.40	-0.27	0.24	0.26	0.75
	Hymenolepis pusilla	*Hymenolepis spinocirrosa*	*Retinometra macrocanthos*	*Microphallus* sp.	*Prosthogonimus anatinus*	*Psilochasmus oxyurus*
NMDS2	-0.73	-0.72	-0.23	0.15	0.18	0.29
	Sphaeridiotrema spp.	*Plenosoma minimum*	*Cotylurus gallinulae hebraicus*	*Capillaria* spp.	*Sobolevicanthus* sp.	*Strongyloides* sp.
NMDS3	-0.59	-0.51	-0.50	0.09	0.11	0.12
	Fimbriaria fasciolaris	*Psilochasmus oxyurus*	*Microphallus pygmaeus*	*Baruscapillaria obsignata*	*Cotylurus gallinulae hebraicus*	*Hymenolepis* sp.
NMDS4	-0.50	-0.43	-0.34	0.16	0.18	0.23

Table 1.6. Permutational multivariate analysis of covariance model variables examined for effects on intestinal helminth infracommunity structure in 130 female Lesser Scaup (*Aythya affinis*) collected from 4 regions of the upper Midwest during the springs of 2014 and 2015.

	df	SS	MS	Pseudo-*F*	*P*
Region	2	23494.0	11747.0	5.5109	0.0001
Year	1	12795.0	12795.0	6.0027	0.0001
Age	1	1937.3	1937.3	0.9089	0.5548
Size	1	3639.5	3639.5	1.7074	0.0523
Residual	114	24300.0	2131.6		

SS, sum of squares; MS, mean square; Size, co-variate created from principle coordinate analysis.

Table 1.7. Mean similarity values (Bray-Curtis similarity index) of intestinal helminth infracommunities from 130 female Lesser Scaup (*Aythya affinis*) between (lower diagonal) and among (shaded, diagonal) 3 regions of collection analyzed in permutational multivariate analysis. Regions were: Upper Mississippi River Valley (UMRV), Central Mississippi River Valley (CMRV), and Illinois River Valley (IRV). Values of the *t*-statistic, with associated nominal *p*-values in parentheses, of pairwise PERMANCOVA tests (upper diagonal).

	IRV	CMRV	UMRV
IRV	38.966	2.3714 (0.001)	2.9112 (0.001)
CMRV	27.442	26.631	1.6025 (0.003)
UMRV	29.930	27.544	32.429

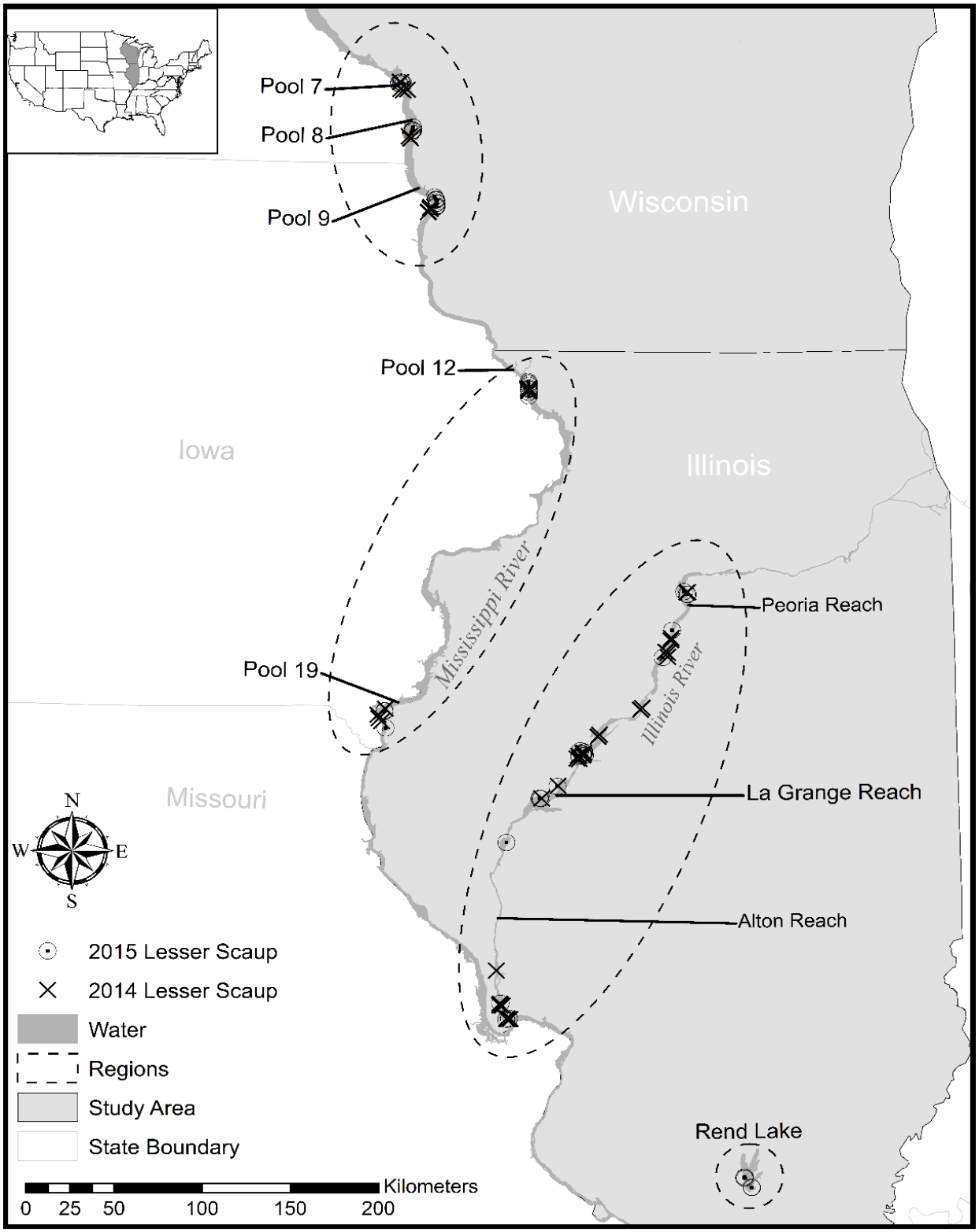

Figure 1.1. Locations of collection sites for female Lesser Scaup (*Aythya affinis*) in the upper Midwest, USA. From top to bottom, regions were: Upper Mississippi River Valley (pools 7-9), Central Mississippi River Valley (pools 12 and 19), Illinois River Valley (IRV [Peoria Reach-Alton Reach], and Southern Illinois (Rend Lake).

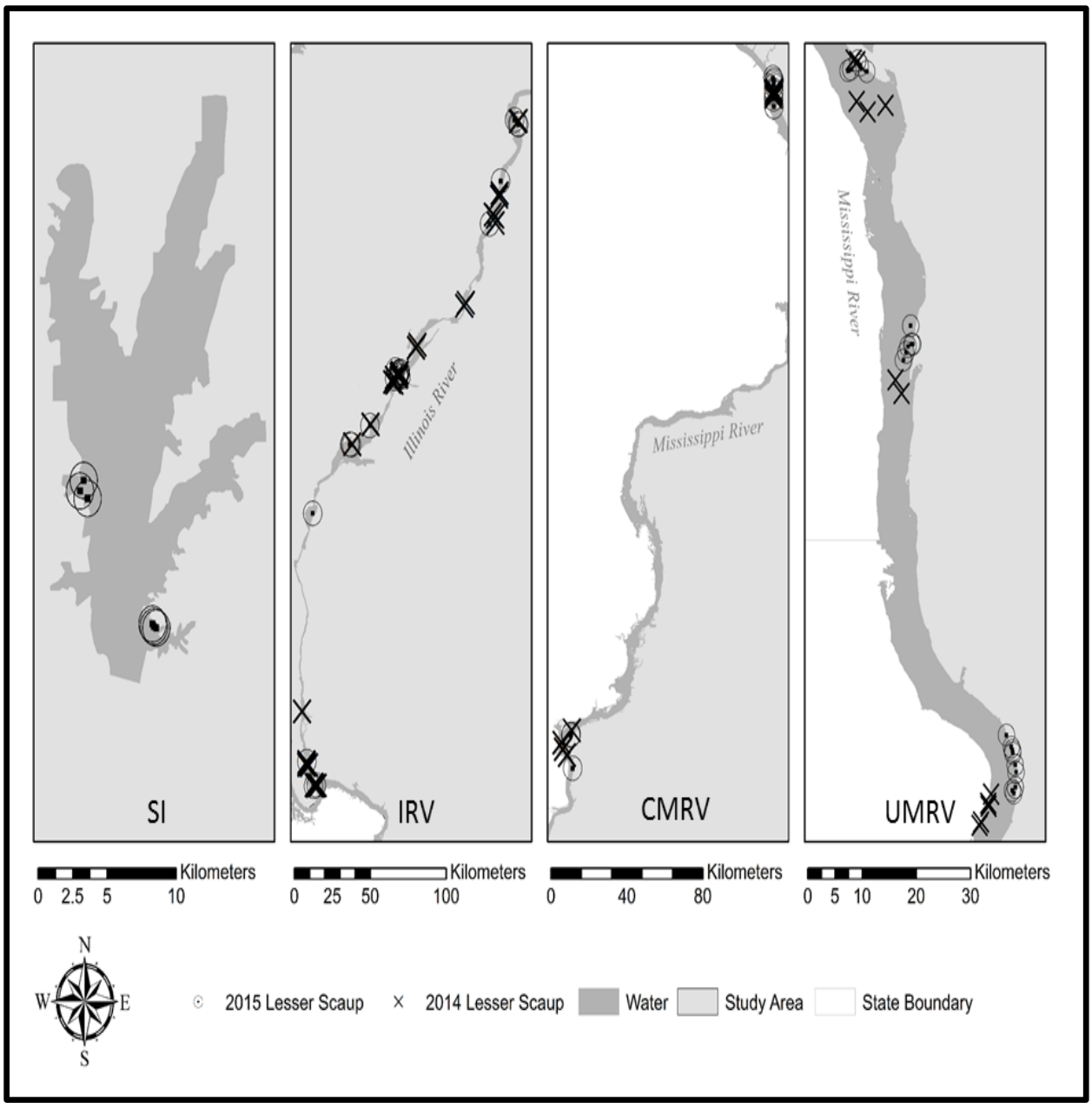

Figure 1.2. A closer view of the locations of collection sites for female Lesser Scaup (*Aythya affinis*) from wetland areas of the upper Midwest. From left to right, regions were: Southern Illinois (SI [Rend Lake]), Illinois River Valley (IRV [Peoria Reach-Alton Reach]), Central Mississippi River Valley (CMRV [pools 12 and 19]), and Upper Mississippi River Valley (UMRV [pools 7-9]).

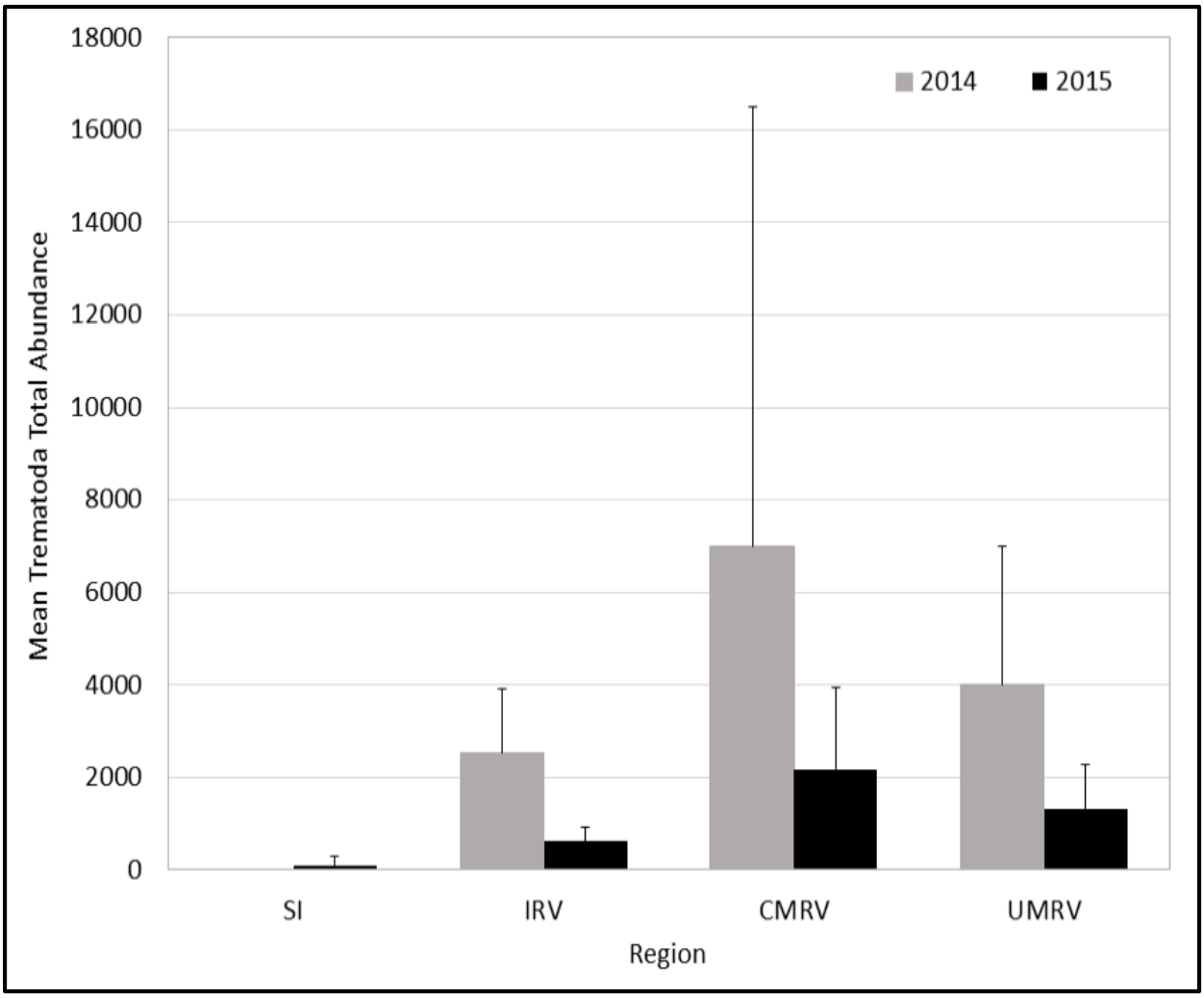

Figure 1.3. Mean abundance (vertical bars) and 95% confidence intervals (solid, capped lines) of intestinal helminths from the class trematoda from 130 female Lesser Scaup (*Aythya affinis*) collected from 4 regions of the upper Midwest, USA, during spring 2014 and 2015, including Southern Illinois (SI), Illinois River Valley (IRV), Central Mississippi River Valley (CMRV), and Upper Mississippi River Valley (UMRV).

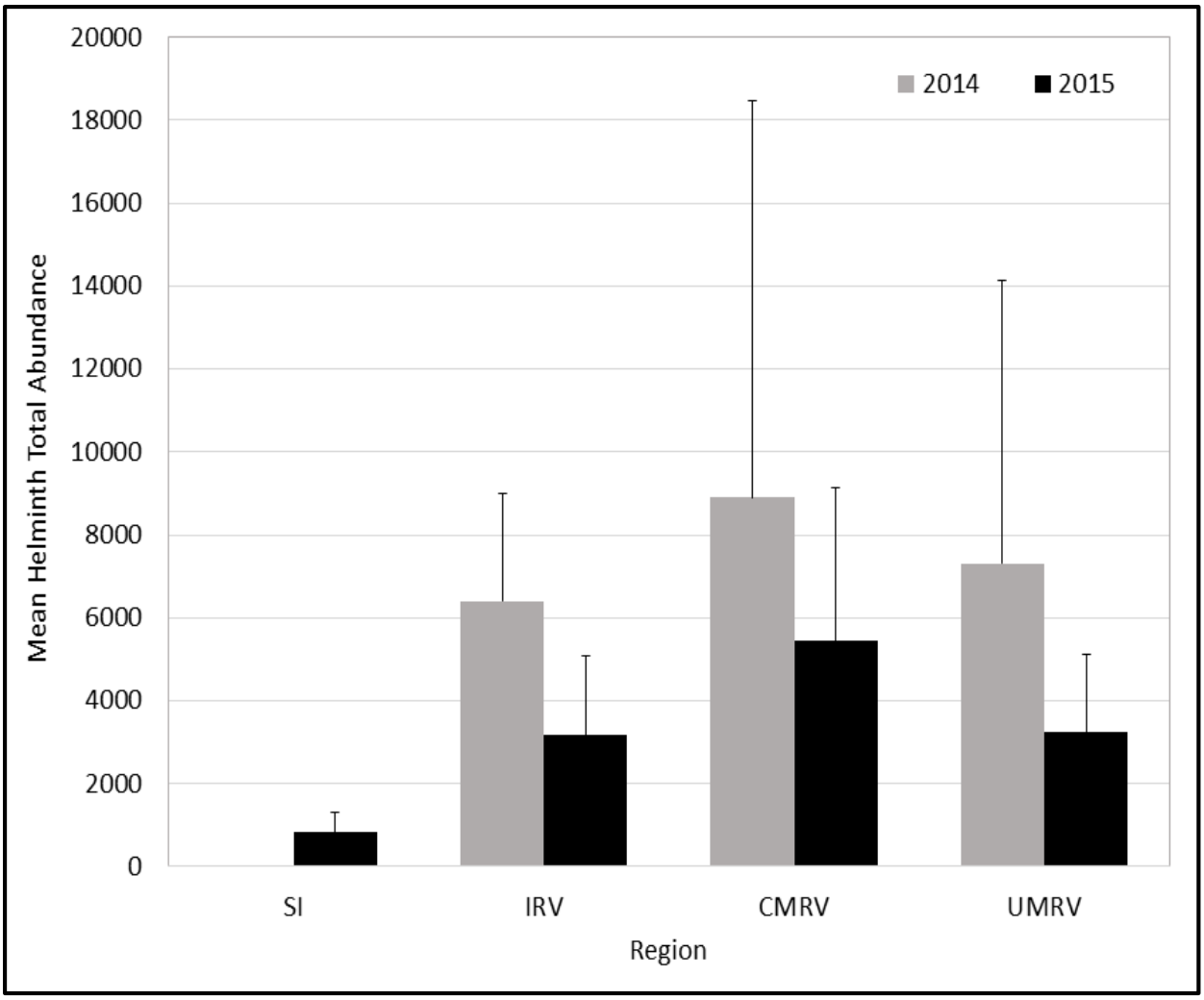

Figure 1.4. Mean total abundance (vertical bars) and 95% confidence intervals (solid, capped lines) of all intestinal helminths from 130 female Lesser Scaup (*Aythya affinis*) collected from 4 regions of the upper Midwest, USA, during spring 2014 and 2015, including Southern Illinois (SI), Illinois River Valley (IRV), Central Mississippi River Valley (CMRV), and Upper Mississippi River Valley (UMRV).

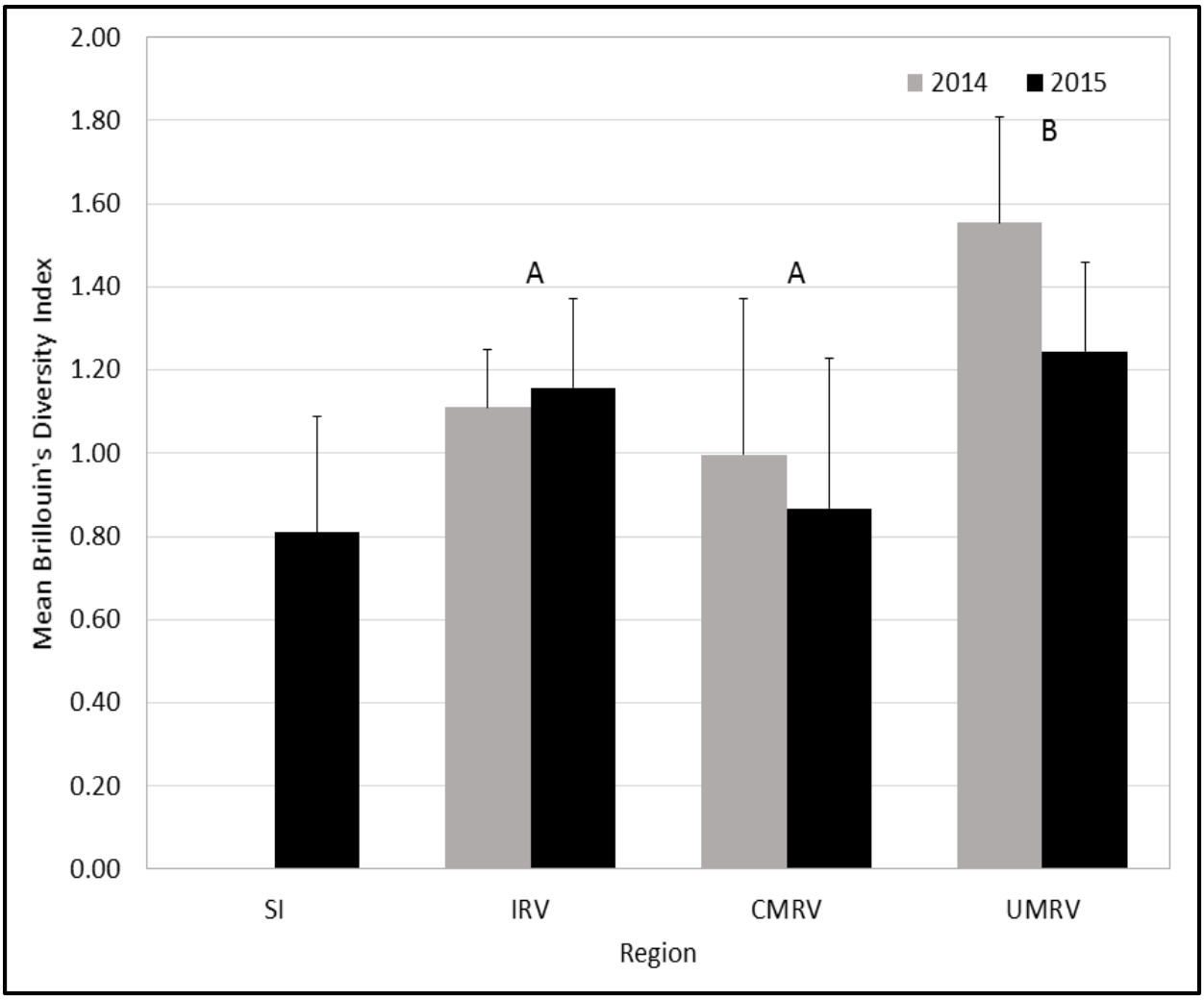

Figure 1.5. Mean Brillouin's species diversity index values (vertical bars) and 95% confidence intervals (solid, capped lines) of intestinal helminths from 130 female Lesser Scaup (*Aythya affinis*) collected from 4 regions of the upper Midwest, USA, during spring 2014 and 2015, including Southern Illinois (SI), Illinois River Valley (IRV), Central Mississippi River Valley (CMRV), and Upper Mississippi River Valley (UMRV). Means not sharing the same letters are significantly different (Tukey's HSD test) according to region ($\alpha = 0.05$). Southern Illinois was excluded from analysis since no Lesser Scaup were collected there in 2014.

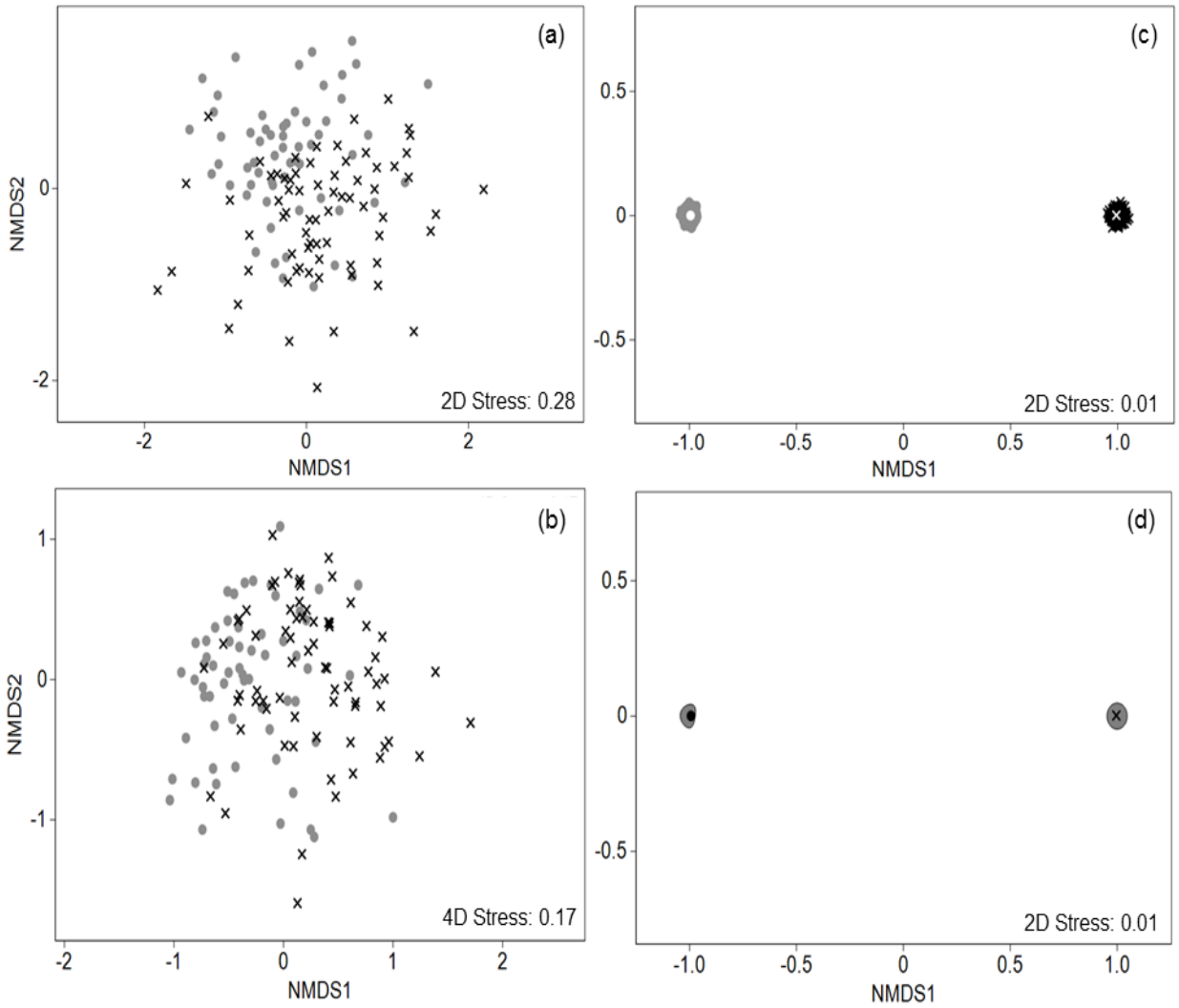

Figure 1.6. Non-metric multidimensional scaling (NMDS) ordination of intestinal helminth communities of 130 female Lesser Scaup (*Aythya affinis*) according to year of collection from 4 regions of the upper Midwest, USA, during spring 2014 and 2015. (a) NMDS plot of 2D NMDS axes 1 and 2. (b) NMDS plot of the 4D axes 1 and 2. (c) NMDS plot of 4D axes 1 and 2 from 100 bootstrap averages (grey circles, 2014; black X's, 2015) and means (white circle, 2014; white X, 2015). (d) NMDS plot of 4D axes 1 and 2 means (black circle, 2014; black X, 2015) with 95% confidence ellipses.

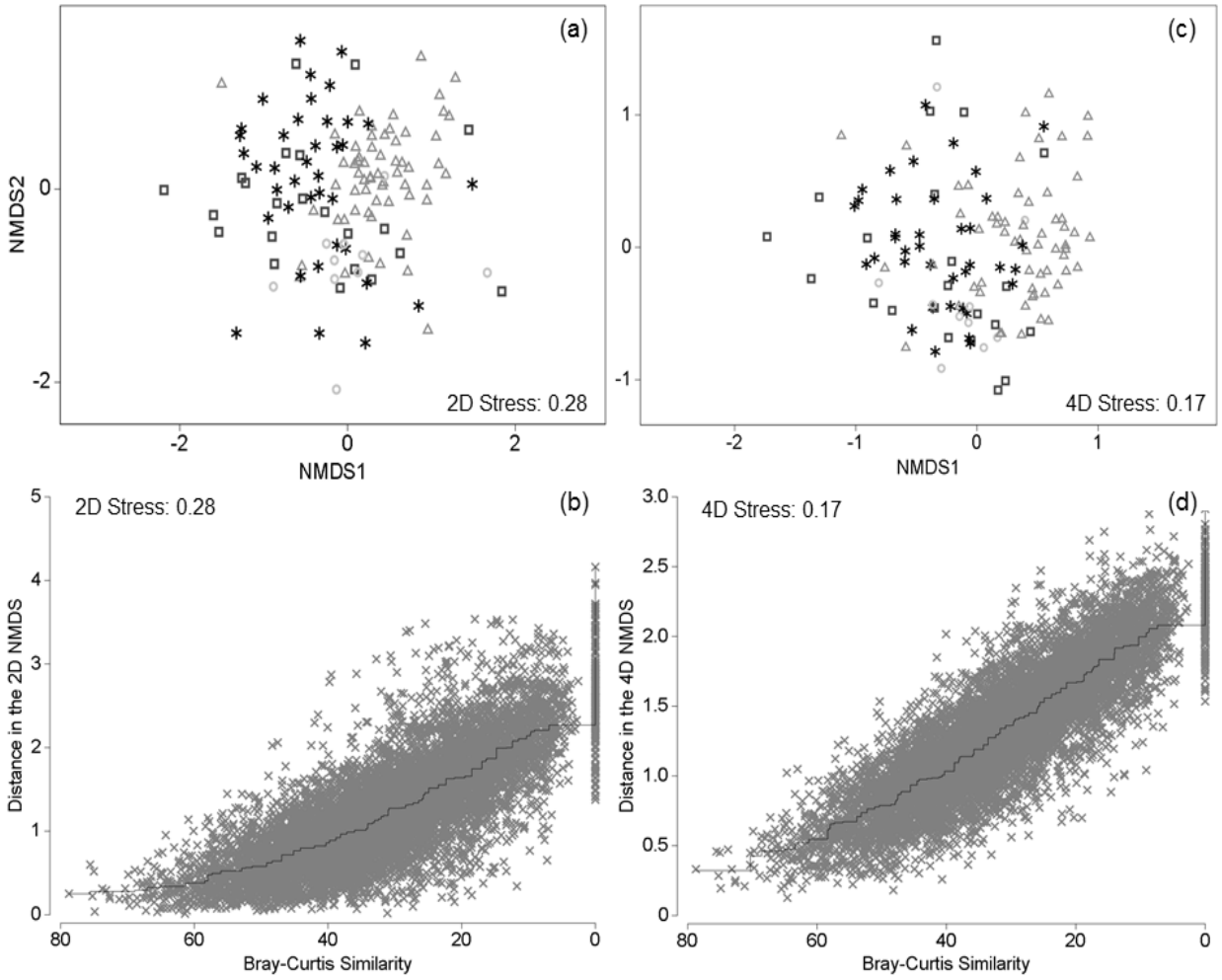

Figure 1.7. Non-metric multidimensional scaling (NMDS) ordination of intestinal helminth communities of 130 female Lesser Scaup (*Aythya affinis*) collected from 4 regions of the upper Midwest, USA, during spring 2014 and 2015, including the Upper Mississippi River Valley (UMRV [black asterisks]), Central Mississippi River Valley (CMRV [open, grey squares]), Illinois River Valley (IRV [open, grey triangles]), and Southern Illinois (SI [open, grey circles]). (a) NMDS plot of 2D NMDS axes 1 and 2. (b) NMDS Shepard diagram in the 2D NMDS. (c) NMDS plot of 4D NMDS axes 1 and 2. (d) NMDS Shepard diagram in the 4D NMDS.

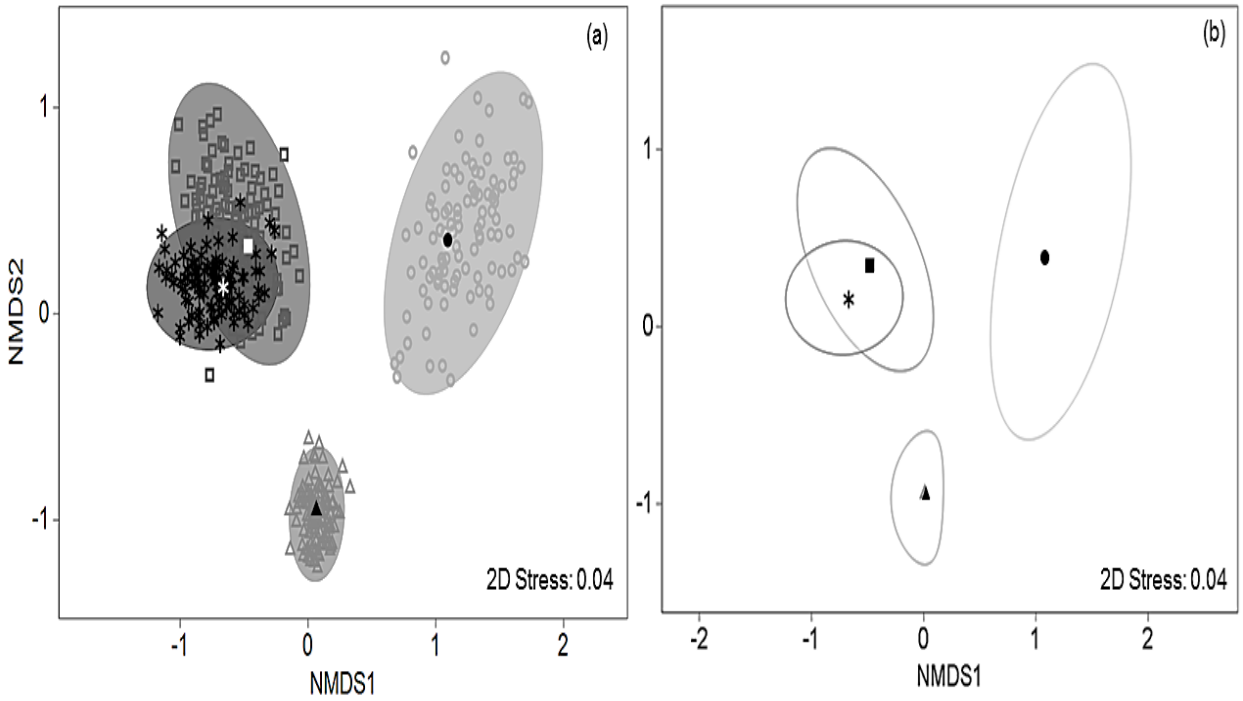

Figure 1.8. Non-metric multidimensional scaling (NMDS) representations of the 4D 100 bootstrap NMDS averages, means, and 95% confidence ellipses for intestinal helminth communities of 130 female Lesser Scaup (*Aythya affinis*) collected from 4 regions of the upper Midwest, USA, during spring 2014 and 2015, including the Upper Mississippi River Valley (UMRV), Central Mississippi River Valley (CMRV), Illinois River Valley (IRV), and Southern Illinois (SI). (a) NMDS ordination of 4D bootstrap averages axes 1 and 2; black asterisks, UMRV bootstrap averages, white asterisks, UMRV mean; open, grey squares, CMRV bootstrap averages, white square, CMRV mean; open, grey triangles, IRV bootstrap averages, black triangle, IRV mean; open, grey circles, SI bootstrap averages, black circle, SI mean. (b) Region means from 100 bootstrap averaging iterations; black asterisks, UMRV mean; black square, CMRV mean; black triangle, IRV mean; black circle, SI mean.

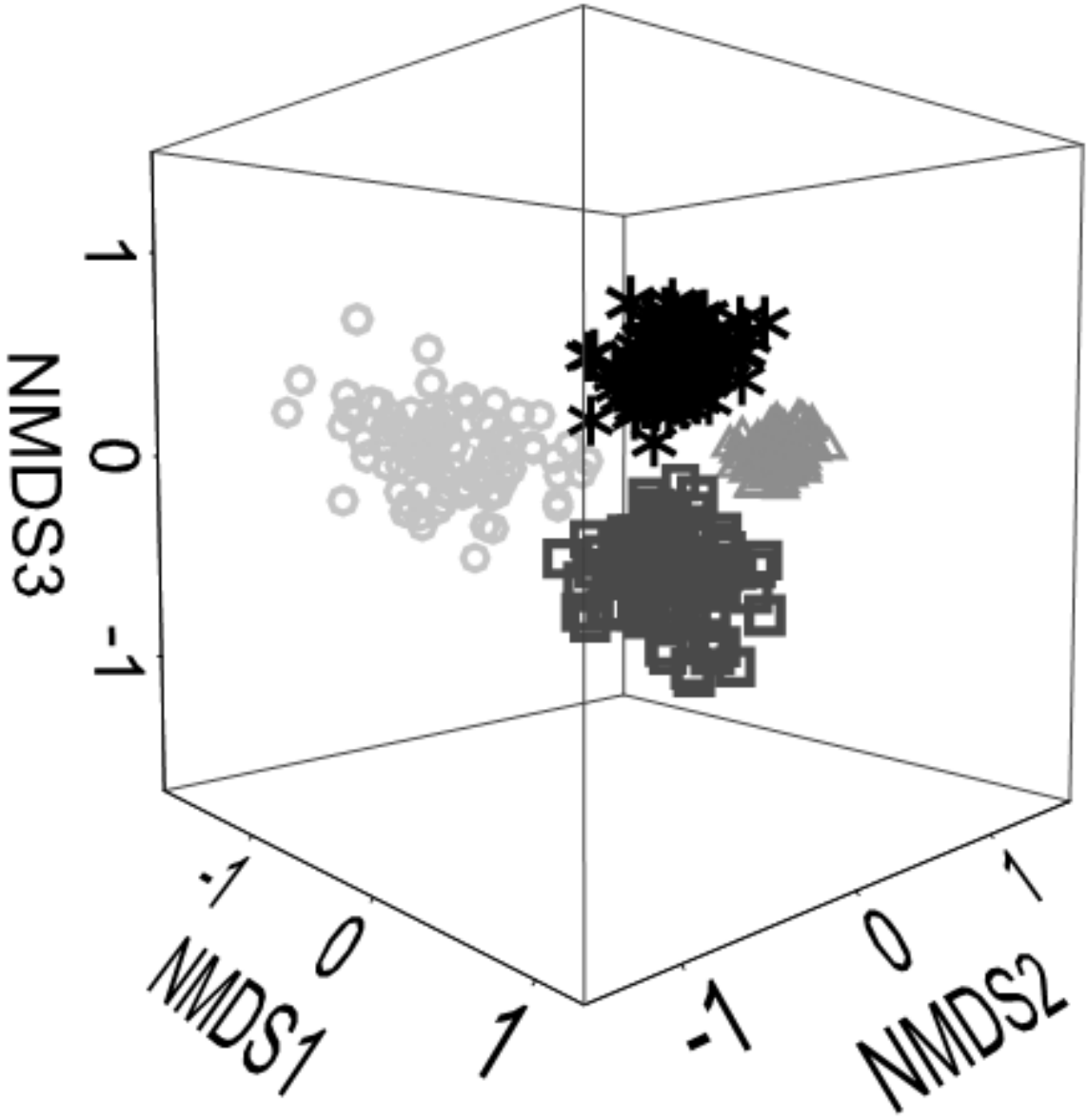

Figure 1.9. 3D representation of the 4D non-metric multidimensional scaling (NMDS) ordination of intestinal helminth communities of 130 female Lesser Scaup (*Aythya affinis*) collected from 4 regions of the upper Midwest, USA, during spring 2014 and 2015, including the Upper Mississippi River Valley (UMRV [black asterisks]), Central Mississippi River Valley (CMRV [open, grey squares]), Illinois River Valley (IRV [open, grey triangles]), and Southern Illinois (SI [open, grey circles]).

CHAPTER 2: ASSOCIATIONS OF INTESTINAL HELMINTH INFECTIONS WITH HEALTH PARAMETERS OF SPRING-MIGRATING FEMALE LESSER SCAUP IN THE UPPER MIDWEST, USA

2.1 INTRODUCTION

Each spring, thousands of Lesser Scaup (*Aythya affinis*) are found dead or dying in wetland areas surrounding the Upper Mississippi River National Wildlife and Fish Refuge (Herrmann and Sorensen 2011; Sandland et al. 2014). These die-offs have been regularly occurring since 2002 due to an introduced snail and two nonindigenous trematode species (Digenea) that utilize it as an intermediate host (Sauer et al. 2007). The Lesser Scaup is a species of conservation concern, and is highly susceptible to infections with these lethal trematodes (Austin et al. 2006; Herrmann and Sorensen 2011). The continental breeding population of Lesser Scaup was estimated to be 8 million in 1972, but declined to 3.2 million by 2006 (Vest et al. 2006; USFWS 2015) and is still approximately 30% below the long-term goal of 6.3 million birds established by the North American Waterfowl Management Plan (NAWMP) (Strand et al. 2008; USFWS 2015). In light of these mortality events, parasitological examinations tasked with identifying associations between intestinal helminths and parameters of Lesser Scaup health are increasingly needed to elucidate how parasites may be contributing to the decline.

Continental populations of Lesser and Greater Scaup (*A. marila*) have experienced a significant decline since the early 1970s. Lesser Scaup (hereafter, scaup unless otherwise specified) constitute approximately 89% of the combined continental scaup population and appear to be the main species in decline (Austin et al. 2000; Afton and Anderson 2001). Several mechanisms including habitat loss and degradation, climate change, pollution, invasive species, predation, and competition have been identified as negatively affecting scaup condition (Afton and Anderson 2001; Anteau and Afton 2004, 2006, 2008a; Strand et al. 2008; Drever et al. 2012).

Anteau and Afton (2004) suggested the Spring Condition Hypothesis (SCH) as the primary cause for scaup pollution declines. The SCH identifies reduced recruitment as a primary cause for population declines and indicates that a lack of adequate, nutritious forage along spring migration corridors in the Mississippi Flyway, particularly the upper Midwest, leads to reduced condition of females during migration towards their breeding grounds. In addition to low quality forage, recent digenean (Class Trematoda) parasitic infections facilitated by the invasive intermediate host, the Faucet Snail (*Bithynia tentaculata*), are to blame for deaths of thousands of scaup and other waterbirds migrating along the upper Mississippi River annually (Sauer et al. 2007; Herrmann and Sorensen 2011; Sandland et al. 2014). These epizootics typically coincide with the energetically demanding migration and pre-breeding periods of scaup and further deplete endogenous nutrient reserves required for migration events and successive breeding efforts (Sauer et al. 2007).

Since its translocation from its native Eurasian waters and introduction into Lake Michigan supposedly by way of cargo ship ballasts in 1871 (Mills et al. 1993), the Faucet Snail's range has spread drastically, and it is now documented in lacustrine and riverine systems stretching from coastal New York to lakes of Montana (Mitchell and Cole 2008). The greatest densities of the snail appear to be located in the upper pools of the Mississippi River (Pools 4-12) and the Great Lakes regions, which are historically important stopover sites for scaup migrating through the Mississippi Flyway (Herrmann and Sorensen 2009; Karatayev et al. 2012; Chapter 1). The Faucet Snail is the primary and secondary intermediate host of *Cyathocotyle bushiensis*, *Sphaeridiotrema pseudoglobulus*, and *Sphaeridiotrema globulus* the digeneans responsible for the large scale die-offs of scaup in the upper Midwest (Sauer et al. 2007; Bergmame et al. 2011; Herrmann and Sorensen 2011; Sandland et al. 2014). *Sphaeridiotrema. pseudoglobulus* and *S. globulus* have been linked to tens of thousands of deaths in 13 species of waterbird throughout the Faucet Snail's range in the upper Midwest since 2002 (Price 1934; Hoeve and Scott 1988; Sauer et al. 2007; Bergmame et al. 2011; Herrmann and Sorensen 2011; Sandland et al. 2014). However,

all parasitological information to date has been collected from dead or moribund scaup limiting the direct link between health and parasite infections.

Scaup become infected with the digeneans after ingesting an infected snail. The infective metacercarial stage of the parasites matures then invades the scaup host's intestinal tract, attaches to intestinal villi, and feeds on blood. Scaup infected with *Sphaeridiotrema* species often develop disease such as gastritis with intestinal lesions including hemorrhage, mucosal sloughing, and fibrinous casts (Hoeve and Scott 1988; Cole and Friend 1999). Those infected with *C. bushiensis* develop typhlitis characterized by sloughing of the mucosal lining of the ceca, and blockage of the lumen with necrotic cores and debris (Gagnon 1990; Cole and Friend 1999). Field signs of infection in scaup include lethargy, listlessness, and the inability to fly or forage (Herrmann and Sorenson 2009). Hoeve and Scott (1988) reported that waterfowl are capable of receiving a lethal dose of these parasites with the ingestion of a single snail, and death typically occurs within three to ten days of ingestion (Herrmann and Sorensen 2011). Sandland et al. (2014) documented a broadening of the second intermediate host competency by *S. pseudoglobulus* into snail species native to the Mississippi River enhancing the potential for transmission to waterfowl during migration periods. Yet, currently only the Faucet Snail is known to serve as the first intermediate host for this digenean.

Other species of intestinal helminths found parasitizing scaup may also negatively impact host condition through direct and indirect means such as reducing foraging ability, increasing predation vulnerability, and lowering reproductive success (Møller 2005). Previous studies have reported scaup to be hosts of more than 40 species of intestinal helminth (Bush and Holmes 1986a,b) many of which are known to be pathogenic to waterfowl (McDonald 1969, 1981; Huffman 2008). However for many of these pathogenic helminths, detailed knowledge of their life cycles and impacts on parameters of scaup health is lacking. A spring-migrating female scaup host in the upper Midwest suffering from high levels of stress and reduced body condition suggested by the SCH may experience increased morbidity from what was once considered to be a relatively tolerable intestinal helminth load (Austin et al. 2000; Anteau

and Afton 2004, 2006, 2008a; Austin et al. 2006). Often, the energetic demands of migration supersede those required to initiate and sustain an immune response against pathogens as energetic reserves must be rationed to facilitate successful migration and subsequent breeding efforts (Hohman 1986; Hanssen et al. 2004). Furthermore, many parasites are capable of evading a host's immune response (Wobeser 2008), and past research with Common Eiders (*Somateria mollissima*) determined that the energetic costs of an immune response may negatively impact survival and is often foregone (Hanssen et al. 2004). The knowledge gap and complexity of host-parasite interactions often leads to parasitism being dismissed as an influential regulator of wildlife populations (Tompkins et al. 2001; Hudson 2005; Lafferty et al. 2006). Yet, the idea becomes plausible when parasitic infections result in the reduction of host survival and/or fecundity in a density dependent manner (Wobeser 2008). An important question remains whether helminths are associated with the reduced condition of scaup, which could contribute to reduced survival and/or breeding propensity, therefore supporting the SCH.

I collected apparently healthy, free-living Lesser Scaup to examine the sub-lethal effects of intestinal helminthiases on body condition and health metrics. The purpose of this work was to identify parameters of scaup health and habitat characteristics that best predicted select measures of parasitic intestinal helminths. Based on prior research, I expected to see negative associations between various helminth parameters and select metrics of health (Hudson and Dobson 1990; Sepúlveda et al. 1994; Hanssen et al. 2004; Shutler et al. 2012). This study established biochemical, hematological, hepatic element, infection (Chapter 1), and morphometric benchmark data for female scaup migrating through the upper Midwest of the Mississippi Flyway, USA, thus building upon previous studies investigating possible factors contributing to the decline of scaup (Austin et al. 2006; Anteau et al. 2007; Pillatzki et al. 2011; Drever et al. 2012).

2.2 MATERIALS AND METHODS

Study area

The study area included traditionally important stopover wetland sites commonly used by scaup during their spring migrations of 2014 and 2015 along the Mississippi and Illinois rivers, in associated wetland complexes in Illinois and southern Wisconsin, and at Rend Lake reservoir in southern Illinois (Figs. 2.1-2). Due to the unpredictable and continuous movement of scaup during migration, appropriate sites were dependent on scaup presence and utilization of wetlands within target areas. Therefore, random sampling was inappropriate for my question, thus, all major stopover sites identified within the study area were sampled. Based on latitude, physiography, and habitat, the study area was broken into the following distinct regions: Southern Illinois (SI [Rend Lake; 38º06'N, 88º58'W], the Illinois River Valley (IRV [Alton Reach – Peoria Reach; 39º17'N, 90º36'W to 41º01'N, 89º25'W; centered, respectively]), the Central Mississippi River Valley (CMRV [Pools 19 and 12; 40º38'N, 91º10'W and 42º24'N, 90º31'W; centered, respectively]), and the Upper Mississippi River Valley (UMRV [Pools 9 – 7; 43º21'N, 91º12'W to 43º55'N, 91º21'W; centered, respectively]).

Field collection

Female scaup were experimentally collected with shotguns from each of the 4 study area regions during 10 February 2014 – 20 April 2014 and 11 March 2015 – 12 April 2015 (n = 130). Emphasis was placed on female scaup because they have the most influence on population dynamics (Cox and Afton 1996; Taylor et al. 2014). Scaup were not collected from the SI region during 2014 due to low abundance and extensive ice coverage, and I excluded birds collected from this region in 2015 from analyses of the hepatic elements and body composition. Prior to collection, undisturbed flocks of scaup were observed to determine if active feeding was occurring as part of a concurrent study of scaup diet composition (Hagy et al. 2015). Each collection site was marked using a handheld global positioning system (GPS) waypoint and given an alpha-numeric identifier. For each scaup, body (bill tip to terminal retrix), culmen, wing

chord, keel, and tarsometatarsus lengths were measured to the nearest 1 mm, and a body mass to the nearest 20 g was obtained using a spring scale (Afton and Ankney 1991; Carney 1992). A second mass (reported herein as mass) was recorded after the removal of the alimentary tract and right liver lobe. Scaup were grouped into two age classes, hatch year (HY) and after hatch year (AHY), according to iris coloration and plumage condition (Trauger 1974; Carney 1992). Five scaup could not be aged because of damage incurred during the collection process; however, missing individuals were classified as AHY birds according to 10,000 Expectation Maximization (EM) iterations performed on scaup morphometric measurements (Clarke et al. 2014). Each specimen was necropsied for tissue sampling within 15 minutes of collection at the site of retrieval. Gross, visual health assessments were made of each individual for external and internal abnormalities. All procedures were approved by the Animal Care and Use Committee at the University of Illinois (protocols # 14295 and 11229). Scaup collection was conducted under Federal Fish and Wildlife Permit # MB145466-1, Illinois Department of Natural Resources Research Permit # SS15-11, and Wisconsin Department of Natural Resources Research Permit # SRL-SOD-005-2013.

Tissue sampling and analysis

I accessed the body cavity of each scaup for tissue collection by cutting the ribcage and moving the keel to one side. Approximately 2 mL of whole blood was obtained via cardiac puncture technique and transferred to a microcentrifuge vial treated with ethylenediaminetetraacetic acid (EDTA). Blood was centrifuged at 1500 x G for 10 minutes, and plasma supernatant was collected for later biochemical assays. The plasma was transferred from its original vial into another vial and frozen in liquid nitrogen. Analytes β–hydroxybutyrate, non-esterified fatty acids, triglycerides, blood urea nitrogen, glucose, albumin, and bilirubin were measured by the University of Illinois Urbana-Champaign's (UIUC) Veterinary Medicine Diagnostic Laboratory with a Beckman Coulter AU680 Chemistry Analyzer and Beckman Coulter reagents (Beckman Coulter, Brea, California, USA). Packed cell volume (PCV) was determined in the field using microcapillary tubes to assess individuals for anemia and polycythemia. The

microcapillary tubes containing 70 µL of fresh, whole blood were centrifuged at 1500 x G for 10 minutes, and a metric ruler was used to determine PCV (amount of blood cells relative to the overall contents of the tube). Blood smears were made on marked glass microscopy slides and after air drying were fixed with a 10 minute methanol bath followed by staining with a Wright-Giemsa stain kit (Fisher Scientific, Pittsburgh, Pennsylvania, USA). Finished slides were sent to the UIUC Veterinary Medicine Diagnostic Laboratory for differential white blood cell (WBC) counts. Each slide was reviewed for proportions (%) of heterophils, lymphocytes, monocytes, eosinophils, and basophils under compound light microscopy at 50x as part of a 100 cell differential count. The heterophil:lymphocyte ratio, an indicator of chronic physiological stress, was obtained for each scaup by dividing heterophil counts by lymphocyte counts (Krams et al. 2012).

I obtained a 2-mL fecal sample directly from the cloaca, placed it into a cryovial, and flash froze the sample in liquid nitrogen for subsequent tests of the stress hormone corticosterone. In the laboratory, cryovials were stored at -80ºC (Herring and Gawlik 2009). Corticosterone extraction and assay protocol were conducted using Corticosterone EIA kits (Enzo Life Sciences, Farmingdale, New York, USA) following manufacturer instructions.

The right liver lobe from each bird was removed and frozen in marked plastic specimen bags. Liver samples (g[dry weight: dw]) were analyzed for (µg/g[dw]) arsenic (As), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), magnesium (Mg), phosphorus (P), lead (Pb), selenium (Se), strontium (Sr), and zinc (Zn) by inductively coupled plasma mass spectrometry at the Eurofins Frontier Global Services Analytical Laboratory (Bothell, Washington, USA) (Levengood 2003).

Following blood and liver collection, the alimentary canal was removed from each scaup and divided into two sections. The portion comprised of the esophagus to the distal gizzard opening was clamped at each end as well as at the junction of the proventriculus and gizzard then injected with and stored in 10% buffered neutral formalin (BNF) as required for diet analysis as part of the concurrent study

(Hagy et al. 2015). The remaining portion (pylorus to the cloaca) was closed off with zip-ties, injected with and stored in 70% ethanol (EtOH), and refrigerated until parasitological examinations could take place in the laboratory between one and four months post collection date.

Once in the laboratory, the intestines were opened longitudinally from the cloaca to the pylorus. Since several species of intestinal helminth occur only in the ceca, each cecum was removed at the ileocecal junction and assessed for helminths under stereomicroscope. The intestinal and cecal lumena were each scraped and rinsed into separate containers. The intestinal ingesta were repeatedly washed and concentrated in a conical beaker using a modified sedimentation technique (Pritchard 1982; Bush and Holmes 1986a). The washed ingesta were then transferred into a 250-mL graduated cylinder, and fresh tap water was added until the mixture equaled 100 mL. The ingesta were mixed into suspension using a magnetic stirrer and stir bar, and a 10% subsample (10 mL) of the ingesta was removed using an aliquot technique (Bush and Holmes 1986a). The aliquot was transferred to a gridded 6 x 6 Petri dish and systematically examined under stereoscope. Recovered helminths were enumerated (total helminths = aliquot helminths x 10) for each scaup and then preserved using 70% EtOH. Nematodes and acanthocephalans were cleared in lactophenol for temporary mounts and identified to the lowest taxon feasible (Sepúlveda and Kinsella 2013). Trematodes and cestodes were stained using either Semichon's acetocarmine stain or Malzacher's staining technique, cleared in xylene, mounted in Canada balsam, and identified to the lowest taxon achievable using morphological characteristics under compound light microscopy.

Proximate analysis of scaup body composition was conducted on the remaining portion of each carcass. Bills, feet, and feathers were first removed from each eviscerated carcass. Then, carcasses were ground, flash frozen in liquid nitrogen, pulverized, and re-frozen in preparation for the analysis of percent body composition of moisture, fat, crude protein, and ash at the UIUC Meat Science Laboratory. Following Novakofski et al. (1989), a 10 g subsample of the 50 g sample of the homogenized, powdered carcass was first oven dried for a 24-hour period to obtain percent moisture. Percent fat was then

determined by extracting lipids from the oven dried sample using an azeotropic mixture of chloroform:methanol, drying the sample, and weighing again. Percent protein was differentiated from the mineral ash percentage by weighting the sample after incineration. The processes was duplicated for each scaup and the results of each trial were averaged together to generate the final body composition percentages.

Metrics of habitat quality included food use, an index of foraging habitat quality based on blood metabolites (daily lipid dynamics; DLD), and food density at foraging locations (kg[dry])/ha) (Anteau and Afton 2008b; Hagy et al. 2015). In addition to DLDs, scaup food use data (count of diet items from invertebrate, plant, vertebrate taxa and aggregate percentage dry mass of the three diet categories [reported herein as invert %, plant %, vert %] found within scaup upper digestive tracts) were included as habitat quality variables (Hagy and Kaminski 2012b). In order to estimate food density nearby collection locations, five soil core samples (benthic) and five water column sweep samples (nektonic) were obtained at each foraging location where scaup were collected; seeds, tubers, and aquatic invertebrates were removed from samples by hand; and all foods were identified to the lowest taxon feasible, weighed, and extrapolated to kg(dry)/ha using standard protocols (Hagy et al. 2015). Food density estimates were adjusted for processing and diet bias if information was available (Hagy et al. 2011, Hagy and Kaminski 2012a).

Statistical analysis

I implemented a multimodel framework within an information theoretic approach using the second-order form of Akaike's Information Criterion (AIC_c) to identify Lesser Scaup health parameter variables considered important in predicting various metrics of helminth infection (Akaike 1974; Burnham and Anderson 2002; Anderson 2010). I used mean imputation to fill in missing data which simultaneously retained important variable information within scaup samples and kept the data consistent across all variables, as is required for multimodal inference (Zar 2010).

I selected seven helminth parameters (herein defined according to Bush et al. 1997) as response variables and formulated biologically plausible models using a variety of potential predictor variables (n = 41). Response variables included Brillouin's species diversity index, total helminth abundance, total cestode abundance, total nematode abundance, total trematode abundance, *Cyathocotyle bushiensis* intensity (of infection), and *Sphaeridiotrema* spp. intensity. Helminth species richness and the total acanthocephalan abundance were not modeled since species richness is similar to Brillouin's species diversity index and acanthocephalan helminths occurred rarely in scaup. The intensities of infection for *C. bushiensis* and *Sphaeridiotrema* spp. were considered from infected scaup since these species are known to be detrimental to scaup health (Cole and Friend 1999). Log transformations ($\log_{10}$ [x + 1]) or negative binomial scaling measures were conducted on response variables where necessary to meet the assumptions of normality and equal variance (Zar 2010). Log transformed data were back-transformed for ease of interpretation and graphical representation. I assessed all predictor variables for correlation using Spearman's rank and excluded those that were highly correlated ($|\rho| > 0.50$) from the same model to avoid multicollinearity. To further reduce the number of possible predictor variables, I built individual models for each predictor variable and compared AIC_c scores between these models and the null model (intercept-only). I excluded those predictors from further analyses which had a greater AIC_c score than the null model due to lack of support (Anderson 2010; Hagy et al. 2010). This method resulted in different health parameters being modeled for each model set.

Within the reduced model set for each dependent variable, I ran all possible independent variable combinations and second order interactions if biologically plausible (PROC GLMMIX in SAS v9.4). Because I was mainly interested in the relative importance of relationships between the various helminth infection metrics and parameters of scaup health, I incorporated the variables year and region of collection as random effects within all candidate models. For candidate models within each model set, I calculated model weight and considered models within seven ΔAIC_c of the top model to be competitive (Arnold 2010; Burnham et al. 2010; Chiavacci et al. 2015). Then, I assessed relative variable importance

within each response variable model set by summing the model weights ($\omega i\ AIC_c$) of each predictor variable across candidate models (Burnham and Anderson 2002; Hagy et al. 2010). Sums of weights ($\sum \omega i$) were calculated and compared across all response variables for interpretation of predictor variable importance. When model selection uncertainty arose, I calculated model-averaged beta-coefficient estimates (β), unconditional standard errors (± SE), and 85% confidence limits (CL), which are more appropriate for *AIC*-based model selection (Arnold 2010; Chiavacci et al. 2015). Competing candidate models were assessed for pretending variables, and candidate models found to contain them were excluded from model ranking and selection as well as model averaging (Anderson 2010; Arnold 2010).

Means, standard deviations (SD), and minimum-maximum values (PROC MEANS in SAS v9.4) were calculated for all biochemical, hematological, hepatic elemental, diet composition, habitat quality, and morphometric data according to year and region of collection as well as scaup age class (HY and AHY). The aforementioned descriptive statistics were also calculated for the pooled data of all 130 scaup samples. These values were evaluated and compared to ranges found in existing literature for Lesser Scaup and/or closely related waterfowl species. Furthermore, helminth taxa prevalence and mean overall food availability (kg[dry]/ha) by region were also calculated and assessed to identify possible contributors influencing scaup condition (Appendix).

2.3 RESULTS

Helminth examination

A total of 647,174 individual intestinal helminths from 40 taxa were found parasitizing 130 female Lesser Scaup in the upper Midwest, USA, during the spring migrations of 2014 and 2015 (Table 2.1). All scaup were infected with 2–23 different helminth taxa. Of the helminths recovered, 44.6% of the individuals from 20 taxa were identified as trematodes, 53.9% of individuals from 14 taxa were cestodes, 1.4% of four taxa were nematodes, and less than 0.05% of helminths recovered belonged to two species of acanthocephala. Cestodes typically occurred at moderate prevalences and mean intensities, whereas

trematodes varied between low and high prevalences and intensities (Table 2.1). Nematodes were generally highly prevalent, but occurred at low mean intensities, and acanthocephalans had low prevalences and low mean intensities (Table 2.1). All helminth species recovered were found to be of reproductively mature stages within scaup hosts, with the exception of the digenean *Echinostoma trivolvis* (a trematode of muskrats [*Ondatra zibethicus*]) and an unidentified juvenile cestode (reported herein as Juvenile).

Associations of helminths and health parameters

After the initial screening, 11 predictor variables remained for inclusion in final models across dependent variable model sets (Table 2.2). The 11 predictor variables retained in final model sets included percent body fat, non-esterified fatty acids, glucose, albumin, bilirubin, blood urea nitrogen, packed cell volume, heterophil:lymphocyte ratio, basophils, monocytes, and DLD.

Percent body fat, non-esterified fatty acids, glucose, albumin, and monocytes were relatively important predictors of Brillouin's diversity index (Table 2.3). Glucose had the highest weight ($\sum\omega i$ = 0.945) and a positive relationship ($\beta = 0.002 \pm 0.001$, 85% CL = 0.001 to 0.014) with the diversity index. The variables albumin ($\sum\omega i = 0.832$, $\beta = -0.240 \pm 0.107$, 85% CL = -0.394 to -0.087), monocytes ($\sum\omega i$ = 0.718, $\beta = -0.033 \pm 0.017$, 85% CL = -0.057 to -0.009), and percent body fat ($\sum\omega i = 0.528$, $\beta = -0.013 \pm$ 0.008, 85% CL = -0.024 to -0.002) also appeared to be important predictors of Brillouin's diversity index, but the relationship was negative. The 85% CLs of NEFA overlapped zero indicating no real effect (Table 2.2; Fig. 2.3).

Only glucose was retained as a predictor of the total helminth abundance (Table 2.2). However, the glucose fixed effect model (ωi = 0.485) ranked below the null model (ωi = 0.515) indicating that more variation in total helminth abundance was explained in the null model (Table 2.4). Beta-estimates showed a positive relationship between glucose and total helminth abundance, yet 85% CLs showed overlap of zero (Table 2.2; Fig. 2.4).

Three health variables were retained as potential predictors of total cestode abundance (Table 2.2). Only the single variable model for DLD ranked above the null model, yet there was high model uncertainty (Table 2.5). Total cestode abundance was negatively related to DLDs ($\sum\omega i$ = 0.670, β = -2.035 ± 0.016, 85% CL = -2.058 to -2.012), which indicated that an increase in cestodes corresponded with a slight decrease in DLD values. The variables bilirubin and the heterophil:lymphocyte ratio were also modeled as predictors of the total cestode abundance, and were positively ($\sum\omega i$ = 0.421, β = 29.409 ± 13.555, 85% CL = 9.890 to 48.928) and negatively ($\sum\omega i$ = 0.180, β = -6.152 ± 3.083, 85% CL = -10.592 to -1.713) associated, respectively (Table 2.2; Fig 2.5).

Bilirubin was the only predictor variable retained for nematode abundance (Table 2.2). Bilirubin accounted for approximately 50% of model weight ($\omega i\ AIC_c$ = 0.505), whereas the null model accounted for the remaining weight (Table 2.6). A negative association was detected between bilirubin and total nematode abundance ($\sum\omega i$ = 0.505, β = -17.218 ± 5.668, 85% CL = --25.380 to -9.056). This negative association indicated that an increase in nematode abundance was associated with a decrease in plasma bilirubin levels.

Total trematode abundance was modeled by the predictor variables non-esterified fatty acids, glucose, albumin, bilirubin, and monocytes. The predictor variable non-esterified fatty acids accounted for the majority of variable weight across candidate models ($\sum\omega i$ = 0.804) followed by albumin ($\sum\omega i$ = 0.550), and the remaining predictor variables which accounted for approximately 26% each (Table 2.2). While model uncertainty was high, six models outranked the null model, and five of those six contained non-esterified fatty acids as an explanatory variable (Table 2.7). Only non-esterified fatty acids (β = -0.846 ± 0.338, 85% CL = -1.332 to -0.360) and albumin (β = -0.776 ± 0.439, 85% CL = -1.398 to -0.133) had consistently negative effects associated with total trematode abundance (Table 2.2; Fig. 2.5).

Cyathocotyle bushiensis intensity was modeled with only the white blood cell predictor, basophils (Table 2.2). Basophils accounted for greater than 50% of model weight ($\omega i\ AIC_c$ = 0.52), while the null

model made up the remainder (Table 2.8). Model averaging indicated a positive association between *C. bushiensis* infection intensity and basophils ($\sum\omega i$ = 0.524, β = 0.429 ± 0.217, 85% CL = 0.117 to 0.741). The association between the response and basophils indicated that increased infection with *C. bushiensis* corresponded with an increased basophil white blood cell response.

Percent body fat, bilirubin, DLD, and packed cell volume were the health parameters remaining for the response variable *Sphaeridiotrema* spp. intensity. All models with the exception of the single effect of percent body fat ranked above the null model (Table 2.9). Packed cell volume contained the greatest weight ($\sum\omega i$ = 0.886) and was negatively associated with *Sphaeridiotrema* spp. intensity (β = -0.060 ± 0.023, 85% CL = -0.091 to -0.028) indicating that increases in the response variable correspond to a decrease in packed cell volume. Bilirubin ($\sum\omega i$ = 0.843) was also negatively associated with increased intensity of *Sphaeridiotrema* spp. (β = -3.243 ± 1.188, 85% CL = -4.953 to -1.533) signifying bilirubin decreases with increased infection intensity. The habitat quality measure, DLD, accounted for a moderate sum of weights ($\sum\omega i$ = 0.536) and showed a negative association with increased *Sphaeridiotrema* spp. infection intensity (β = -0.023 ± 0.009, 85% CL = -0.036 to -0.010). Percent body fat was positively associated with *Sphaeridiotrema* spp. intensity; however, the beta-estimates of percent body fat had CLs that overlapped zero (Table 2.2; Fig. 2.6).

2.4 DISCUSSION

Eleven of the health parameters used as indicators of scaup condition, including measures of body condition, hematologic homeostasis, and immune condition, were associated with helminth infection metrics. Given the decline in body condition and foraging success (e.g., lower fat levels and DLD scores) in the northernmost region where invasive trematodes occurred, certain helminth taxa and assemblage characteristics may impact scaup survival and reproductive success by reducing body condition during spring migration (Tompkins and Begon 1999; Wobeser 2008; Vandegrift et al. 2008). Furthermore, the combination and interaction of parasitic helminth effects with other exogenous and endogenous stressors

experienced by scaup may further contribute to and/or encourage a reduced state of overall condition during spring migration. The observed associations between health parameters and helminth metrics herein explicate the sublethal impacts the intestinal helminths impose on their scaup hosts. Investigations of the sublethal effects of parasitism on the survival and fecundity of free ranging host species have received more attention from the scientific community within recent years (Hudson 1986; Hudson et al. 1999; Tompkins et al. 2001; Stein et al. 2002; Vandegrift et al. 2008). Such studies have challenged the traditional paradigm that parasites only induced mortality on already sick individuals and are not able to significantly influence wild population dynamics (Lack 1954; Lafferty and Kuris 2005). Increasing evidence suggests that even moderate infections with gastrointestinal helminths may induce morbidity, thus reducing host body condition and fecundity (Tompkins et al. 2001; Stein et al. 2002; Vandegrift et al. 2008). If such effects were to be imposed on scaup by infections of intestinal helminths, then it would be entirely plausible that parasitism (whether directly or indirectly) may be a significant factor contributing to the SCH proposed by Anteau and Afton (2004). My findings offer another plausible mechanism underlying the SCH due to a helminth-influenced reduction in scaup condition.

The trends of several health parameters observed in this study are reflective of other declining waterbird populations. Hollmén et al. (2001) suggested declines of Common Eider populations in Finland are a result of diminished food resources impacting female Common Eider condition prior to and during their nesting cycle. Female Common Eiders were unable to obtain the food resources needed to successfully nest or initiate immune responses, which often resulted in increased disease and mortality rates. Additionally, Hollmén et al. (2001) observed notable differences between stable and declining eider populations, with blood metabolite (e.g., free fatty acids and albumin) concentrations and fat reserves being lower and indicative of starvation, lowered condition, and presumably increased susceptibility to pathogens in the latter. Additionally, helminth infections in other sea duck species have resulted in negative effects similar to those I observed in scaup (i.e., reduced condition, increased morbidity, dietary shifts) and mortality events (Skerratt et al. 2005). The helminths found parasitizing scaup have developed

adaptations to exploit resources quickly and efficiently given the relative brevity of the definitive host's presence during the spring migration (Holt and Boulinier 2005; Baldassarre 2014). The die-offs occurring in scaup, as well as those reported of sea ducks, highlight the ability of parasitic infections to act in concert with other environmental and anthropogenic stressors to exacerbate the decline of stressed waterfowl populations.

Percent body fat was negatively associated with Brillouin's diversity index. Body fat is important for female scaup due to the energetically demanding temporal proximity of spring migration and reproduction (Anteau and Afton 2004; Stafford et al. 2014). The observed association between percent body fat and Brillouin's species diversity index most likely coincided with the fact that helminth species diversity was highest in the UMRV region of the study area. Intestinal helminth assemblages tended to shift with increasing latitude from a cestode species dominated assemblage to one dominated by trematode species, whereas nematodes and acanthocephalans occurred at comparatively low intensities and increased with latitude regardless of the dominant taxa. The observed shifts in helminth assemblage composition are suspected of being influenced by geography and intermediate host presence (Chapter 1), but interspecific competition amongst helminth taxa may also explain these shifts. Such competitive interactions for nutrients within scaup hosts, may incur other energetically demanding responses (e.g., immune response, increased foraging, etc.) thus further depleting fat reserves (Shutler et al. 2012).

Contrary to my initial predictions, an effect of percent body fat was not detected within the remaining helminth metrics. I expected to see a negative effect within total helminth abundance and total trematode abundance based on information within the existing literature and the fact that the helminth species causing the scaup mortality events are themselves trematodes (Huffman 2008; Shutler et al. 2012). A significant effect between percent body fat and total abundance of all helminths and/or trematodes may not have been detected for two reasons. First, no two helminth species impact their host in the same manner. Simultaneous infections of multiple helminth species may lead to a race to exploit

host nutrients (Shutler et al. 2012), therefore masking the effects of a single species. Second, additional factors may have been affecting fat deposition such as poor forage availability and/or quality.

Increasingly, hematological examinations are being adopted for wildlife health assessments due to the ease of sampling and limited stress placed on individuals (Wobeser 2008). Of the 14 health parameters derived from scaup blood samples, eight appeared as relatively important predictors of certain helminth infection metrics, and five of those eight were the plasma metabolites non-esterified fatty acids, glucose, albumin, bilirubin and blood urea nitrogen. Nutrients needed by helminths for growth and propagation are most often sequestered from a host by passive absorption or directly by ingestion (and sometimes both). Helminth species that acquire nutrients directly by ingesting host blood derive the minerals and serum factors needed for their survival at the expense of the host, hence the importance of plasma metabolites observed in this study (Asahi 2009). Additionally, several of the helminths observed in this study cause losses of nutrients from the blood stream by burrowing into tissues causing hemorrhage (Cole and Friend 1999). Fluctuations of non-esterified fatty acid levels in the blood stream may be indicative of starvation and/or malabsorption, and inadequate forage for scaup is suspected in most regions of the Midwest during spring migration (Harris 2009; Straub et al. 2012; Hagy et al. 2015). During fasting or starvation periods, non-esterified fatty acids are released into the blood stream for oxidization into usable energy forms in the muscles and liver after being cleaved from lipid storages. Non-esterified fatty acids were negatively associated with increased helminth species diversity and increased total trematode abundance indicating a body fat mobilization response by scaup due to a negative energy balance or other stressors.

Glucose is a fundamental carbon source for lipid creation in birds and is used to replenish glycogen reserves or is converted to fat deposits (Fesler and Peterson 2013). Additionally, glucose levels increase after feeding but may also increase following corticosterone production during periods of acute stress (Wendelaar Bonga 1997; Wagner and Congleton 2004; Harris 2009). Although a myriad of factors may contribute to increased glucose levels, hyperglycemia in birds is often a result of high levels of

endogenous glucocorticoids released as a stress response (Harris 2009; Harr 2009), and increasing intensities of helminth infections during the pre-breeding spring migration period is likely stressful for female scaup. Glucose levels were greater in regions with higher mean latitudes, which may coincide with both recent foraging and/or situations stressful to scaup. Glucose levels were positively associated with increases in the helminth species diversity index. The most substantial association of glucose (based on weight of evidence) was seen in Brillouin's species diversity index. As previously discussed, the northern regions of the study area were more species rich than the southern regions. Also, infections with the introduced trematode species, *C. bushiensis* and *Sphaeridiotrema* spp., became more common as latitude increased and may have contributed to increases in glucose as a stress response. Determining whether increased glucose levels in scaup infected with diverse helminth infracommunities were due to recent feeding events or recent stress events was not possible in this study, and it is likely that both events were occurring simultaneously. The removal of nutrients either by direct nutrient loss or by malabsorption caused by parasite facilitated tissue damage will often induce stress on the host, which in turn may initiate a hunger response (Wagner and Congleton 2004). Therefore, scaup with infections of certain helminth species may respond as if nutritionally deprived and forage more intensively thus increasing glucose concentrations.

The transport protein albumin primarily regulates osmotic pressure in birds and acts as a protein transport for various nutrients (Harris 2009). Low levels of albumin in an individual are often associated with serious conditions such as malnutrition, intestinal malabsorption, liver failure, renal failure, septicemia, and subacute to chronic blood loss (Harr 2009). Like non-esterified fatty acids, albumin has been identified as a necessary blood serum factor for parasite cellular growth and propagule development (Asahi 2009). Albumin levels were inversely associated with increasing helminth species diversity and total trematode abundance. Reductions in albumin often coincide with intestinal malabsorption, malnutrition, and blood loss which are conditions typically brought on by the intestinal helminths found in this study (Harr 2009). Additionally, the negative associations of albumin with Brillouin's diversity

index of helminths and total trematode abundance may allude to a competition for host nutrients within the helminth infracommunity. Mean concentrations of albumin were lowest in the UMRV region of the study area. Again, this may be due to the introduced trematodes species previously discussed acquiring nutrients from a host by consuming blood and causing blood loss, therefore directly reducing albumin content within the host.

Bilirubin is produced in the liver and altered levels suggest the lack or excess of certain essential elements (Harris 2009); however, hepatic element concentrations assessed herein were consistent with those previously reported in waterfowl (Takekawa et al. 2002; Custer et al. 2003; Pillatzki 2007; Pillatzki et al. 2011). Additionally, bilirubin has also been linked to impaired liver function and may fluctuate based on indirect impacts associated with the liver (e.g., nutrient deprivation and elevated glucocorticoid levels) (Harr 2009). Low bilirubin concentrations in the blood have been used as a marker of anti-oxidant status and as a predictor of certain diseases (e.g., liver disease, protein-losing enteropathy, nutrient deficiencies, etc.) (Shcherbinina 2007; Harr 2009). Furthermore, bilirubin is involved with the metabolic breakdown of senescent red blood cells and may cause tissue damage in its unconjugated form (Harr 2009). Bilirubin appeared as an important positively related predictor of total cestode abundance but was negatively associated with total nematode abundance and *Sphaeridiotrema* spp. intensity with a negative association. The cestodes encountered in this study typically do not migrate into tissue and absorb nutrients passively across their tegument (McLaughlin 2008), whereas the nematodes and *Sphaeridiotrema* spp. often do migrate through host tissue resulting in blood loss, inflammation, and sometimes secondary infections (Cole and Friend 1999; Yabsley 2008). Although birds produce little bilirubin due to a lack of biliverdin reductase, it would seem that existing concentrations are most likely affected by blood loss and malnutrition brought on by decreased intestinal absorption from certain helminth infections (Fairbrother et al. 1990).

Blood urea nitrogen is a measurement of urea in the blood and is associated with renal function and the hydration status of an individual, thus elevated blood urea nitrogen implies dehydration (Harris

2009). Several trematode species found parasitizing scaup have been shown to cause dehydration in infected individuals (e.g., *C. bushiensis*), but dehydration can result from migratory events as well therefore obscuring the significance between blood urea nitrogen and helminth infections (Hoeve and Scott 1988; Gagnon et al. 1993; Harris 2009). However, I detected no association between blood urea nitrogen and trematode total abundance.

Packed cell volume is a quick, practical measurement used to test a subject for anemia or dehydration (Campbell 1988). However, packed cell volume varies in waterfowl according to sex, age, time of year, geography, and period of the annual cycle (Kocan 1972; Shave and Howard 1976). Mean values of packed cell volume were typically within the normal ranges based on the literature, but packed cell volumes did appear as an important negatively associated predictor of *Sphaeridiotrema* spp. Due to the fact that many of the helminths found in scaup (especially *C. bushiensis* and *Sphaeridiotrema* spp.) feed on blood directly, damage mucosal linings, and cause blood loss through the intestinal villi, it is not unreasonable to anticipate that packed cell volume would be negatively associated in birds with increasing infections (Roscoe and Huffman 1982; Gagnon 1990; Cole and Friend 1999). *Sphaeridiotrema* spp. have been documented in causing blood loss and subsequent death by hypovolemic shock in waterbird hosts (Cole and Friend 1999); therefore, packed cell volume was expected to appear as an important predictor on *Sphaeridiotrema* spp. infection intensity. A packed cell volume may be useful as a future diagnostic measure of *Sphaeridiotrema* spp. intensity of infection due to the easy of obtainment and relationship with this trematode.

The leukocyte profile of a bird is another useful tool for diagnosing pathogenic infections (Campbell 1988). Heterophils are the most common avian WBC found in the peripheral circulatory system and are involved with inflammatory responses and immune initiated reactions to parasitic infections (Mitchell and Johns 2008). Lymphocytes also frequently increase in response to foreign antigens (Krams et al. 2012). The ratio of heterophils to lymphocytes has been used as an indicator of morbidity and chronic stress in birds due to its sensitivity to natural stressors and indicative of elevated

glucocorticoid levels (Krams et al. 2012). I expected the heterophil:lymphocyte ratio to appear throughout several of the response model sets, yet it only appeared as a negatively associated predictor of total cestode abundance with a minimal sum of weights. I suspect that the high turnover of several of the intestinal helminth species made observations of sustained, chronic immune responses a rare occasion in scaup. The heterophil:lymphocyte ratio has been associated with stress incurred from chronic parasitic infections in several avian species (Cirule et al. 2012), but an acute-phase immune response has been documented to vary according with pathogen identity (Coon et al. 2011; Krams et al. 2012). Unlike the acanthocephalan and nematode species, cestodes were typically present at comparatively greater prevalences and intensities throughout all regions of the study area; therefore, suggesting that cestodes may have been the only taxa capable of altering the heterophil:lymphocyte ratio consistently. Furthermore, the trematodes causing visible signs of pathology and die-offs of scaup are associated with acute infections and pathology (Hoeve and Scott 1988; Cole and Friend 1999). Therefore, I suspect that the heterophil:lymphocyte ratio was not a viable predictor of trematode infections in scaup, particularly infections with the non-indigenous species, *C. bushiensis* and *Sphaeridiotrema* spp., which are associated with high scaup mortality. Two other WBC metrics appeared in several model sets. Basophils are WBCs that increase in response to inflammation and suppress parasitic worm infections (Harris 2009). Basophils were positively related with increasing infections of *C. bushiensis*, which is appropriate given this trematode typically infects the ceca and causes typhlitis (Gagnon 1990; Cole and Friend 1999). Monocytes are large, phagocytic WBCs whose cytoplasmic granules contain lysozymes used to destroy invading organisms and chemicals involved in mediating inflammation (Campbell 1988; Mitchell and Johns 2008). Monocytes appeared as important predictors of the Brillouin diversity index. Like percent body fat, glucose, and albumin, the association of monocytes and the species diversity index may be due in part to the competition of helminths within the scaup intestinal tract.

Fecal corticosterone levels are have been associated with prolonged nutritional and environmental stress in avian species (Herring et al. 2011; Taylor et al. 2014). Although brief increases in corticosterone

concentrations may positively benefit reproductive investment (Love et al. 2014), birds chronically exposed to amplified levels may display decreased condition and suppressed immune response due to complex interactions of corticosterone, contaminants, and metabolic processes (Pollock and Machin 2009). I initially hypothesized that corticosterone would be positively associated with increased infections of intestinal helminths. Contrary to expectations, corticosterone was not found to be a significant predictor for any of the helminth metrics examined. It is more likely that corticosterone fluctuations were attributable to scaup diet and migration events than helminth stressors. As with the heterophil:lymphocyte ratio, long-term stressors measured as a part of this study intending to serve as predictors of infections with certain helminths were most likely obscured by the acute pathology exhibited by infections with the introduced trematodes.

Metrics of food availability and DLD values have been used to determine the condition and quality of wetlands (Anteau and Afton 2011; Hagy et al. 2015). Briefly, DLDs are a measure of the inverse relationship exhibited between the plasma metabolites β-hydroxybutyrate and triglycerides. β-hydroxybutyrate is reflective of active metabolic catabolism, and increased levels signify a breakdown of lipid reserves. Alternatively, triglycerides are obtained during digestion and increased levels in the blood represent lipid deposition within scaup. Anteau and Afton (2008b) developed the DLD index to represent daily mass changes in scaup, therefore creating a representative measurement of foraging habitat quality. Furthermore, forage biomass availability and DLDs are considered direct measurements of habitat quality and differ from indirect measures (i.e., waterfowl abundance and behavior [Hagy and Kaminski 2012b], physical habitat attributes [Straub et al. 2012], juxtaposition of habitats [Webb et al. 2010]) by providing an immediate assessment of a habitat's influence on scaup body condition (Anteau and Afton 2011). Total cestode abundance and *Sphaeridiotrema* spp. intensity were both negatively associated with DLDs, signaling either a lack of quality forage in areas associated with greater amounts of these helminths, reduced nutrient absorption in infected scaup, or both. The negative energy balance experienced in the

UMRV coupled with a higher intermediate host and helminth diversity could affect scaup percent fat reserves making a significant effect more detectable.

The DLD index appeared as a moderate, negatively associated predictor of *Sphaeridiotrema* spp. intensity of infection and total cestode abundance. With regards to *Sphaeridiotrema* spp. intensity, the DLD relationship suggests that scaup with infections of this introduced trematode are not depositing lipids. In support of this, the UMRV had the second greatest amount of food biomass availability within the study area after the IRV. However, the UMRV displayed the lowest mean DLD value of any region, thus indicating a negative energy balance for foraging scaup. Although food density was second greatest in the UMRV, invertebrates composed the majority of food items in this region, whereas positive DLD values were generally associated with collection sites that contained extensive moist-soil vegetation and plant foods (Hagy et al. 2015). In this study area, invertebrates are often likely to be intermediate hosts for most of the helminths observed herein. In a sea duck mortality study, Skerratt et al. (2005) suggested that waterfowl in compromised health may switch to more abundant, non-preferred prey when experiencing starvation or reduced food availability. Additionally, the introduced Faucet Snail is robust to extreme changes in temperature, chemical disinfection, and pH; is highly competitive; and the snail has been reported of displacing many of the native gastropods of the upper Midwest that scaup historically preyed upon (Mitchell and Cole 2008). This suggests that the type of food available in the UMRV was likely more detrimental than beneficial to scaup due to the presence of *C. bushiensis* and *Sphaeridiotrema* spp. in the region. *Cyathocotyle bushiensi*s always co-occurred with *Sphaeridiotrema* spp.; however, infections of *Sphaeridiotrema* spp. often occurred without *C. bushiensis*. These digeneans attach directly to intestinal epithelium, burrow through tissue, and feed on blood. Infections in scaup often result in severe enteritis and hemorrhage, endogenous reserve depletion, skeletal muscle atrophy, and death (Hoeve and Scott 1988; Gagnon et al. 1993). Scaup infected with the introduced trematodes were typically in poorer condition when compared to scaup lacking the introduced trematodes based on the health parameters examined herein (i.e., reduced lipid reserves and packed cell volume). Therefore, the

metabolites associated with the DLD index may be influenced more substantially by the metabolic interferences attributed to the cachexia and malabsorption caused by infections of *Sphaeridiotrema* spp. Unlike *Sphaeridiotrema* spp., I assume that the DLD index was associated with total cestode abundance because these helminths passively absorb nutrients from intestinal ingesta, inflict less damage to host tissues, and do not directly impact blood parameters as observed with *Sphaeridiotrema* spp. infections. Furthermore, cestodes often occurred at greater prevalences and comparatively greater intensities across all regions of the study area regardless of the habitat quality indicated by DLDs thus suggesting less intermediate host specificity.

Conclusions and management implications

Global climate change and the continued degradation of habitat throughout the range of Lesser Scaup has been linked to reduced reproductive success and survivability (Austin et al. 2000; Anteau and Afton 2008a; Drever et al. 2012). Scaup migrations are the most protracted of any diving duck and their arrival on the breeding grounds corresponds to historical peak abundances of amphipods, a primary prey item (Bellrose 1976; Anteau and Afton 2004, 2006, 2008a; Wobeser 2008; Baldassarre 2014). Anthropogenic changes to the landscape and climate are increasingly uncoupling these evolutionary relationships, and the effects of this altered environment on scaup health are unknown. Clearly, the effects of changes in the quantity and quality of food items, contaminant loads, habitat availability and quality, and parasitic infections may be additive and likely impact scaup survival and reproduction as described by the SCH. I acknowledge that although not examined herein, scaup collected in this study could be exhibiting decreased condition due to factors beyond the scope of this project (e.g., viral, bacterial, fungal, and other parasitic pathogens). Nevertheless, I report findings considering the full weight of evidence and maintain that pathogenesis is likely influenced by nutritional status of the host and by environmental stress (Connors and Nickol 1991; Shutler et al. 2012).

Currently, no practical/feasible control measures of helminth infections exist for free ranging waterfowl populations (Wobeser 1997; Vest 2002), but offsetting the negative impacts of intestinal helminths may be possible through habitat restoration, expansion, and improvement (Stafford et al. 2014). Given the temporal proximity of the Lesser Scaup spring migration and breeding season, energy rich forage items (e.g., moist-soil seeds and vegetation) are of the utmost importance. Management activities emphasizing the production carbohydrate-rich plant foods during the spring via wetland creation and/or enhancement may alleviate some of the limitations scaup populations are currently experiencing (Stafford et al. 2014). The information presented herein may be applicable to various waterbird species of conservation concern that are experiencing population wide reductions in reproductive success and survival. Moreover, these results may further be used to identify waterfowl areas in most need of conservation efforts and rehabilitation from wildlife researchers and managers.

Although parasites do negatively impact hosts by direct and indirect means, it is more likely that a combinations of factors are contributing to a reduction in scaup condition and subsequent reproductive efforts. Furthermore, intestinal helminth infections may intensify in compromised individuals who are unable to ameliorate pathogenic effects due to lack of appropriate nutrition along spring migration corridors (Wobeser 2008). My research has provided evidence of associations between parasitic helminth infections and health of free-ranging scaup and established a set of biochemical, hematological, hepatic element, and morphological data. This information can be compared to data from dead individuals collected following mortality events in areas where parasite infection rates are typically high (e.g., Pools 7-9 of the Mississippi River) or captive individuals used in experiments to determine effects of parasites on health and reproduction. Future research should focus on controlled experimentation to identify links between parasitic infections and impacts on various parameters of scaup health and reproduction.

Given the diversity of helminths found parasitizing scaup and previous documentation of their effects on hosts, I expected more health variables to appear as important predictors of helminth abundance, intensity, and diversity. Generally, intestinal helminth infections directly and/or indirectly

deprive a host of nutrients vital to continued homeostasis. The complexity of abiotic and biotic interactions are a persistent problem in ecology that often mask or convolute seemingly simple interpretations. In addition, the mechanisms of parasite-host dynamics are little understood, influenced by a myriad of factors, and range in effect from no observable pathology to host death, making examinations that much more difficult to assess (Wobeser 2008). Lafferty et al. (2006) and Wobeser (2008) have criticized prior research for suggesting that parasites have minimal impact on wild bird populations, but the sublethal and indirect effects of helminthic infections examined herein may lead to reduced fecundity and thus are likely to have an effect at the population level therefore contributing to the SCH. Considering the sheer diversity of helminths known to infect waterfowl (i.e., more than 525 trematode species, 260 cestode species, 200 nematode species, and 50 acanthocephala species [McDonald 1969, 1986, 1988]) coupled with the fact that a single scaup may host thousands of helminths at once and show no signs of illness is a testament to the resilience of this species. However, scaup migrating through the upper Midwest are experiencing reduced condition purportedly due to a variety of factors (Anteau and Afton 2004; Vest et al. 2006; Anteau and Afton 2008a,b), and thus parasite burdens that were once tolerable may be increasingly pathogenic.

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2.6 TABLES AND FIGURES

Table 2.1. Prevalence (P; % infected) and mean intensity of infection (MI) of the intestinal helminth species in 130 female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest, USA, during spring 2014 and 2015.

Helminths											
Acanthocephala	P (%)	MI	Cestoda	P (%)	MI	Nematoda	P (%)	MI	Trematoda	P (%)	MI
Corynosoma constrictum	3.8	19.4	*Anatinella* sp	1.5	90	*Baruscapillaria obsignata*	42.3	10.2	*Apatemon gracilis*[a]	25.4	261.9
Polymorphus marilis	6.2	20.5	*Dicranotaenia coronula*[a]	41.5	60	*Capillaria anatis*	58.5	11	*A. burti*	3.8	16.6
			Diorchis sp 1	36.2	184.3	*C. spinulosa*	1.5	3	*Cotylurus cornutus*[a]	2.3	13.3
			Diorchis sp 2	29.2	89.3	*Capillaria* spp	26.2	6.1	*C. flabelliformis*[a]	7.7	81.7
			Echinocotyle rosseteri	15.4	717.7	*Strongyloides* sp[a]	40	144.3	*C. gallinulae hebraicus*	25.4	53.2
			Fimbriaria fasciolaris[a]	37.7	488.5				*Cotylurus* spp[a]	30.8	93.2
			Fimbriariodes intermedia	7.7	38.2				*Cyathocotyle bushiensis*[a]	10.8	27.7
			Hymenolepis megalops[a]	3.8	119.2				*Diplostomum phoxini*	8.5	38.6
			H. pusilla	76.2	1344.8				*Echinoparyphium recurvatum*[a]	61.5	1248.3
			H. spinocirrosa	81.5	1195.6				*Echinostoma trivolvis*[a,b]	31.5	260.6
			H. tuvensis	25.4	390.4				*Maritrema* sp 1[a]	6.1	184.3
			Hymenolepis spp[a]	30	201.4				*Maritrema* sp 2[a]	3.8	13.8
			Retinometra macrocanthos[a]	32.3	225.5				*Microphallus oblonga*	23.1	596.4
			Sobolevicanthus sp[a]	22.3	104				*M. pygmaeus*[a]	6.9	7088.6
			Juvenile[c]	1.5	495.5				*Microphallus* spp[a]	13.1	1485.2
									Notocotylus sp[a]	8.5	8.8
									Paramonostomum sp[a]	3.1	6.8
									Plenosoma minimum	17.7	1584.5
									Prosthogonimus anatinus	2.3	7.3
									Psilochasmus oxyurus[a]	33.1	38.2
									Sphaeridiotrema spp[a]	30	394
									Zygocotyle lunata[a]	31.5	7.6

[a]Species that are known to be pathogenic to waterfowl species (McDonald 1969, 1986; Huffman 2008; McLaughlin 2008). [b]Species reported to be zoonotic. [c]Juvenile refers to a cestode specimen where all individuals were underdeveloped and unidentifiable.

Table 2.2. Variables used to predict metrics of intestinal helminth infections from 130 female Lesser Scaup (*Aythya affinis*) collected during spring migrations of 2014 and 2015 through the upper Midwest, USA. Values reported for each predictor variable from top to bottom include: sum of weights ($\sum\omega i$); beta-estimate ± unconditional standard error (β ± SE); 85% confidence limits (LCL to UCL). Total abundance refers to all helminths or those of a specific taxa regardless of whether or not Lesser Scaup where infected. *Cyathocotyle bushiensis* and *Sphaeridiotrema* spp. are expressed as intensity of infection. Total helminth abundance, total abundance of cestoda, and total abundance of nematoda were first $\log_{10}$ transformed, and values reported here have been back-transformed.

[a]Predictor Variable	Response Variable						
	Brillouin's Diversity Index	Total Helminth Abundance	Total Cestode Abundance	Total Nematode Abundance	Total Trematode Abundance	*Cyathocotyle bushiensis*	*Sphaeridiotrema* spp.
Fat (%)	0.528 -0.013 ± 0.008 (-0.024 to -0.002)	-	-	-	-	-	0.221 0.014 ± 0.043 (-0.166 to 0.032)
NEFA (mEq/L)	0.414 -0.111 ± 0.087 (-0.237 to 0.014)	-	-	-	0.804 -0.846 ± 0.338 (-1.332 to -0.360)	-	-
GLU (mg/dL)	0.945 0.002 ± 0.001 (0.001 to 0.004)	0.485 0.005 ± 0.005 (-0.002 to 0.012)	-	-	0.260 0.002 ± 0.004 (-0.004 to 0.007)	-	-
ALB (g/dL)	0.832 -0.240 ± 0.107 (-0.394 to -0.087)	-	-	-	0.550 -0.766 ± 0.439 (-1.398 to -0.133)	-	-
BIL (mg/dL)	-	-	0.421 29.409 ± 13.555 (9.890 to 48.928)	0.505 -17.218 ± 5.668 (-25.380 to -9.056)	-	-	0.0.843 -3.243 ± 1.188 (-4.953 to -1.533)
BUN (mg/dL)	-	-	-	-	0.258 -0.044 ± 0.109 (-0.201 to 0.112)	-	-
PCV (%)	-	-	-	-	-	-	0.886 -0.060 ± 0.023 (-0.091 to -0.028)
HL Ratio	-	-	0.180 -6.152 ± 3.083 (-10.592 to -1.713)	-	-	-	-
BAS (%)	-	-	-	-	-	0.524 0.429 ± 0.217 (0.117 to 0.741)	-

Table 2.2. Continued

[a]Predictor Variable	Response Variable						
	Brillouin's Diversity Index	Total Helminth Abundance	Total Cestode Abundance	Total Nematode Abundance	Total Trematode Abundance	*Cyathocotyle bushiensis*	*Sphaeridiotrema* spp.
MON (%)	0.718 -0.033 ± 0.017 (-0.057 to -0.009)	-	-	-	0.257 -0.023 ± 0.060 (-0.109 to 0.064)	-	-
DLD	-	-	0.670 -2.035 ± 0.016 (-2.058 to -2.012)	-	-	-	0.536 -0.023 ± 0.009 (-0.036 to -0.010)

[a]Fat = fat % body composition; NEFA = non-esterified fatty acids; GLU = glucose; ALB = albumin; BIL = bilirubin; BUN = blood urea nitrogen; PCV = packed cell volume; HL Ratio = heterophil:lymphocyte ratio; BAS = basophil % of 100 cell white blood cell differential; MON = monocyte % of 100 cell white blood cell differential; DLD = daily lipid dynamics.

Table 2.3. Rankings of competing models (ΔAIC_c<7) according to Akaike's Information criterion for small sample sizes (AIC_c) with the number of parameters (K), model weights ($\omega i\ AIC_c$) and deviance (Dev) for predicting the Brillouin's species diversity index of intestinal helminths using measures of health from 130 female Lesser Scaup (*Aythya affinis*) collected during 2014 and 2015 spring migrations through the upper Midwest, USA.

Model[a]	*K*	AIC_c	ΔAIC_c	$\omega i\ AIC_c$	Dev
Fat + GLU + ALB + MON	8	172.76	0.00	0.26	157.84
GLU + ALB + MON	7	173.28	0.52	0.20	160.60
GLU + ALB	6	174.85	2.09	0.09	164.37
Fat + NEFA + GLU + ALB	8	175.23	2.47	0.08	160.31
NEFA + GLU + ALB	7	175.37	2.61	0.07	162.68
Fat + NEFA + GLU + MON	8	176.24	3.48	0.05	161.32
GLU + MON	6	176.35	3.59	0.04	165.87
Fat + GLU + ALB	7	176.85	4.09	0.03	161.94
Fat + NEFA + GLU	7	177.22	4.46	0.03	165.22
NEFA + GLU + MON	7	177.31	4.55	0.03	164.63
Fat + GLU + MON	7	177.32	4.56	0.03	162.40
Fat + ALB + MON	7	178.12	5.36	0.02	165.44
NEFA + GLU	6	178.66	5.90	0.01	168.18
Fat + NEFA + ALB + MON	8	179.47	6.71	0.01	164.56
ALB + MON	6	179.51	6.75	0.01	169.03
Null	4	188.73	15.97	0.00	180.41

[a]Covariates include: Fat = fat % of Lesser Scaup body composition; GLU = glucose (mg/dL); ALB = albumin (g/dL); NEFA = non-esterified fatty acids (mEq/L); MON = monocyte % of 100 cell white blood cell differential; Null = intercept only model with Year + Region as random effects.

Table 2.4. Rankings of competing models (ΔAIC_c<7) according to Akaike's Information criterion for small sample sizes (AIC_c) with the number of parameters (K), model weights ($\omega i\ AIC_c$) and deviance (Dev) for predicting total helminth abundance using measures of health from 130 female Lesser Scaup (*Aythya affinis*) collected during 2014 and 2015 spring migrations through the upper Midwest, USA.

Model[a]	K	AIC_c	ΔAIC_c	$\omega i\ AIC_c$	Dev
Null	4	473.79	0.00	0.51	465.47
GLU	5	473.91	0.12	0.49	463.42

[a]Covariates include: GLU = glucose (mg/dL); Null = intercept only model with Year + Region as random effects.

Table 2.5. Rankings of competing models (ΔAIC_c<7) according to Akaike's Information criterion for small sample sizes (AIC_c) with the number of parameters (K), model weights ($\omega i\ AIC_c$) and deviance (Dev) for predicting total cestode abundance using measures of health from 130 female Lesser Scaup (*Aythya affinis*) collected during 2014 and 2015 spring migrations through the upper Midwest, USA.

Model[a]	K	AIC_c	ΔAIC_c	$\omega i\ AIC_c$	Dev
DLD	5	549.38	0.00	0.48	541.06
Null	4	551.67	2.29	0.15	545.48
BIL	5	552.04	2.66	0.13	543.72
DLD + HL	6	552.45	3.07	0.10	539.77
BIL + DLD + HL	7	553.31	3.93	0.07	538.39
HL	5	554.09	4.71	0.05	543.61
BIL + HL	6	554.78	5.40	0.03	542.09

[a]Covariates include: BIL = bilirubin (mg/dL); DLD = daily lipid dynamics; HL = heterophil:lymphocyte ratio from 100 cell white blood cell differential; Null = intercept only model with Year + Region as random effects.

Table 2.6. Rankings of competing models (ΔAIC_c<7) according to Akaike's Information criterion for small sample sizes (AIC_c) with the number of parameters (K), model weights ($\omega i\ AIC_c$) and deviance (Dev) for predicting total nematode abundance using measures of health from 130 female Lesser Scaup (*Aythya affinis*) collected during 2014 and 2015 spring migrations through the upper Midwest, USA.

Model[a]	K	AIC_c	ΔAIC_c	$\omega i\ AIC_c$	Dev
BIL	5	476.14	0.00	0.50	469.94
Null	4	476.18	0.04	0.50	472.08

[a]Covariates include: BIL = bilirubin (mg/dL); Null = intercept only model with Year + Region as random effects.

Table 2.7. Rankings of competing models (ΔAIC_c<7) according to Akaike's Information criterion for small sample sizes (AIC_c) with the number of parameters (K), model weights ($\omega i\ AIC_c$) and deviance (Dev) for predicting total trematode abundance using measures of health from 130 female Lesser Scaup (*Aythya affinis*) collected during 2014 and 2015 spring migrations through the upper Midwest, USA.

Model[a]	K	AIC_c	ΔAIC_c	$\omega i\ AIC_c$	Dev
NEFA + ALB	6	2099.28	0.00	0.26	2086.60
NEFA	5	2099.54	0.26	0.23	2089.05
NEFA + GLU	6	2101.53	2.25	0.08	2088.85
NEFA + BUN	6	2101.69	2.41	0.08	2089.01
NEFA + MON	6	2101.69	2.41	0.08	2089.01
ALB	5	2101.84	2.56	0.07	2091.35
Null	4	2103.52	4.24	0.03	2095.20
ALB + BUN	6	2103.63	4.35	0.03	2090.95
ALB + MON	6	2103.67	4.39	0.03	2090.99
GLU + ALB	6	2103.99	4.71	0.02	2091.30
BUN	5	2104.95	5.67	0.02	2094.47
GLU	5	2105.04	5.76	0.01	2094.56
Mon	5	2105.16	5.88	0.01	2094.67
ALB + BUN + MON	7	2105.69	6.41	0.01	2090.77
GLU + ALB + BUN	7	2105.82	6.54	0.01	2090.91
GLU + ALB + MON	7	2105.84	6.56	0.01	2090.92

[a]Covariates include: ALB = albumin (g/dL); BUN = blood urea nitrogen (mg/dL); GLU = glucose (mg/dL); NEFA = non-esterified fatty acids (mEq/L); MON = monocyte % of 100 cell white blood cell differential; Null = intercept only model with Year + Region as random effects.

Table 2.8. Rankings of competing models (ΔAIC_c<7) according to Akaike's Information criterion for small sample sizes (AIC_c) with the number of parameters (K), model weights ($\omega i\ AIC_c$) and deviance (Dev) for predicting *Cyathocotyle bushiensis* intensity of infection using measures of health from 130 female Lesser Scaup (*Aythya affinis*) collected during 2014 and 2015 spring migrations through the upper Midwest, USA.

Model[a]	K	AIC_c	ΔAIC_c	$\omega i\ AIC_c$	Dev
BAS	5	112.48	0.00	0.52	104.08
Null	4	112.67	0.19	0.48	107.58

[a]Covariates include: BAS = basophil % of 100 cell white blood cell differential; Null = intercept only model with Year + Region as random effects.

Table 2.9. Rankings of competing models (ΔAIC_c<7) according to Akaike's Information criterion for small sample sizes (AIC_c) with the number of parameters (K), model weights ($\omega i\ AIC_c$) and deviance (Dev) for predicting *Sphaeridiotrema* spp. intensity of infection using measures of health from 130 female Lesser Scaup (*Aythya affinis*) collected during 2014 and 2015 spring migrations through the upper Midwest, USA.

Model[a]	*K*	*AICc*	Δ*AICc*	*ωi AICc*	Dev
BIL + PCV	6	503.95	0.00	0.29	494.78
Fat + BIL + DLD + PCV	8	506.49	2.54	0.08	491.87
BIL + DLD	6	506.69	2.74	0.07	497.52
PCV	5	506.93	2.98	0.07	500.24
DLD + PCV	6	507.84	3.89	0.04	498.66
Fat + PCV	6	509.05	5.10	0.02	499.87
Fat + BIL + DLD	7	509.11	5.16	0.02	497.29
DLD	5	510.26	6.31	0.01	504.26
Fat + DLD + PCV	7	510.48	6.53	0.01	498.66
Null	4	519.35	15.40	0.00	512.67

[a]Covariates include: Fat = fat % of Lesser Scaup body composition; BIL = bilirubin (mg/dL); DLD = daily lipid dynamics; PCV = packed cell volume (%); Null = intercept only model with Year + Region as random effects.

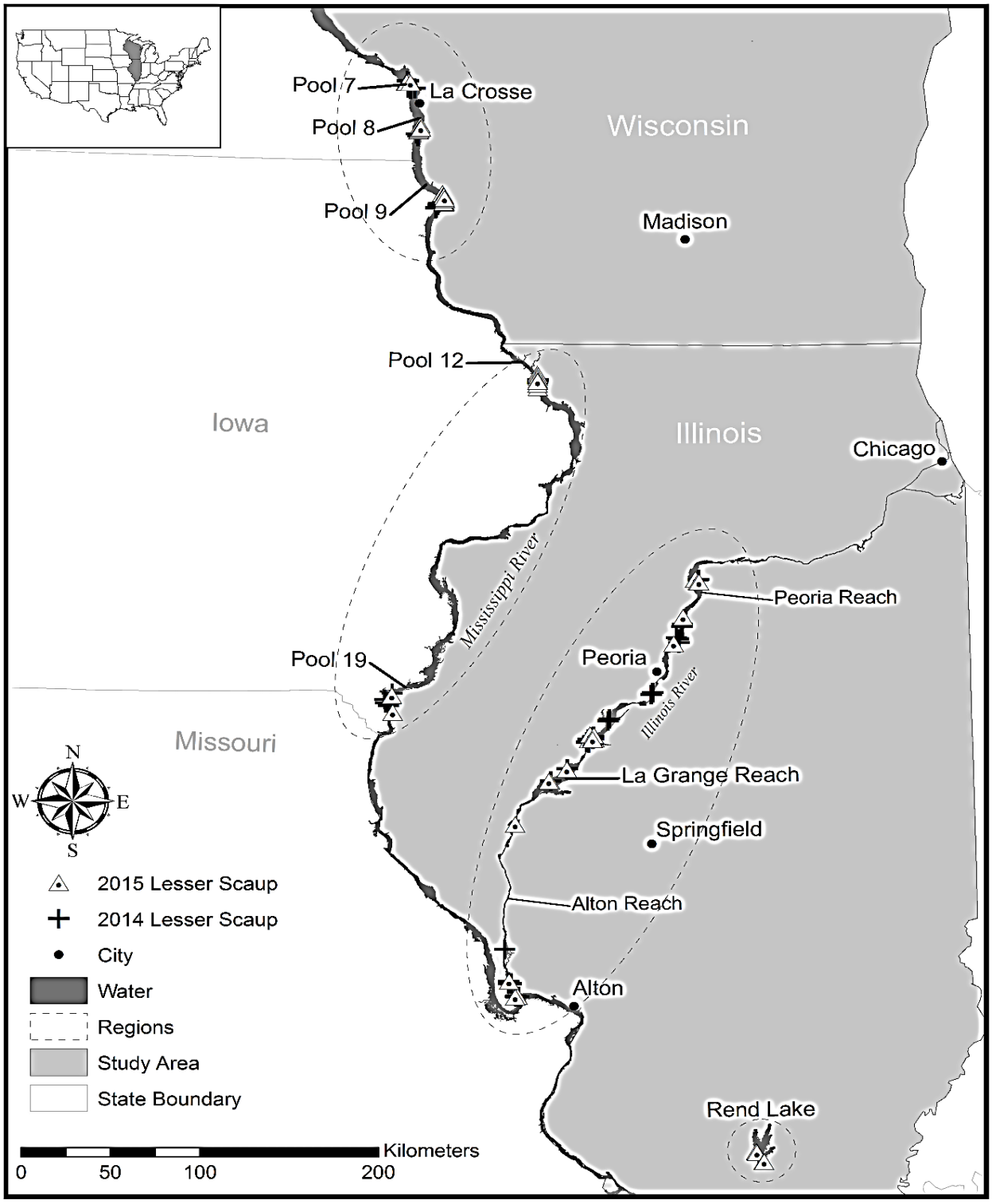

Figure 2.1. Locations of collection sites for female Lesser Scaup (*Aythya affinis*) in the upper Midwest, USA. From top to bottom, regions were: Upper Mississippi River Valley (pools 7-9), Central Mississippi River Valley (pools 12 and 19), Illinois River Valley (IRV [Peoria Reach-Alton Reach], and Southern Illinois (Rend Lake).

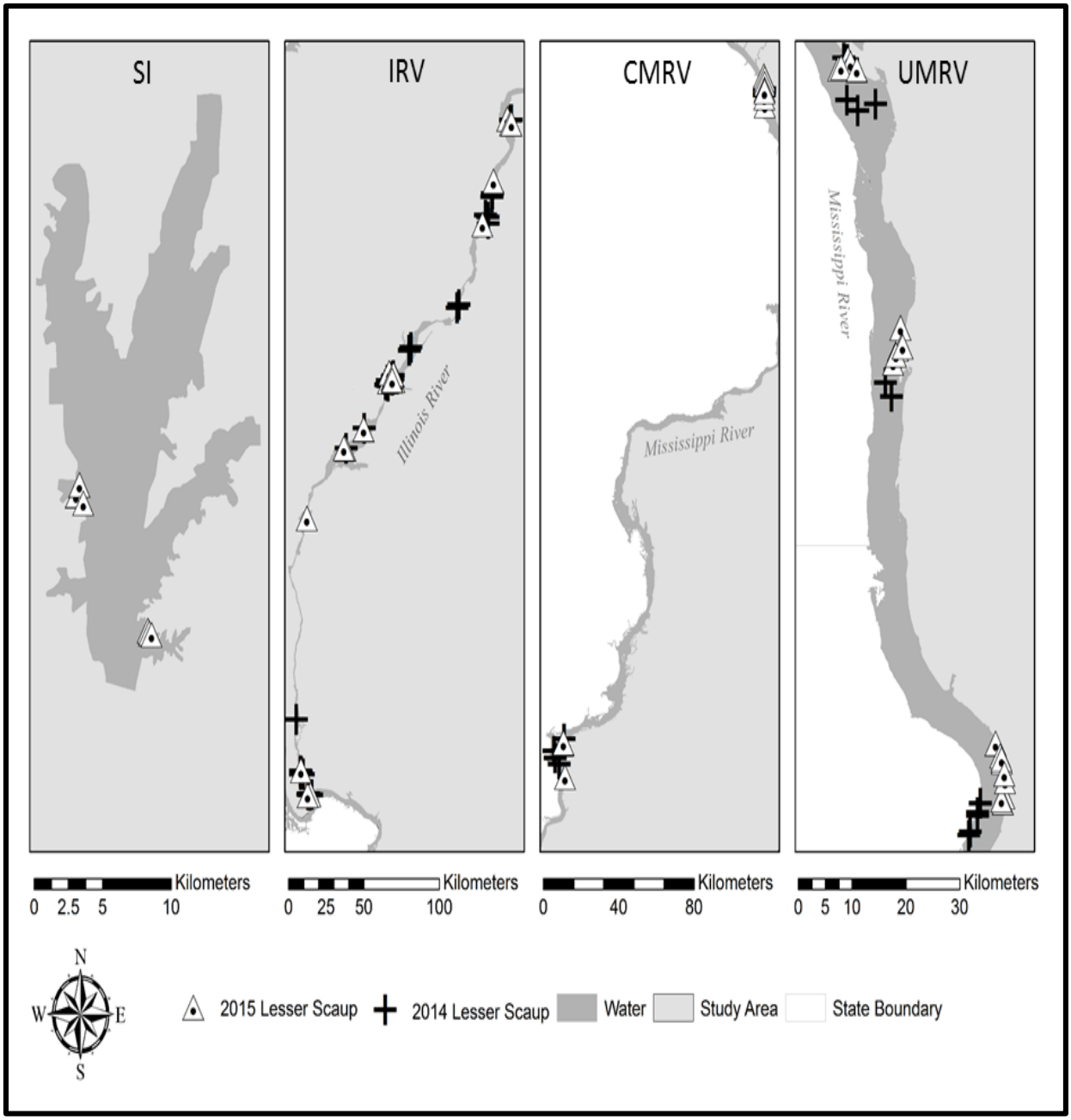

Figure 2.2. A closer view of the locations of collection sites for female Lesser Scaup (*Aythya affinis*) from wetland areas of the upper Midwest. From left to right, regions were: Southern Illinois (SI [Rend Lake]), Illinois River Valley (IRV [Peoria Reach-Alton Reach]), Central Mississippi River Valley (CMRV [pools 12 and 19]), and Upper Mississippi River Valley (UMRV [pools 7-9]).

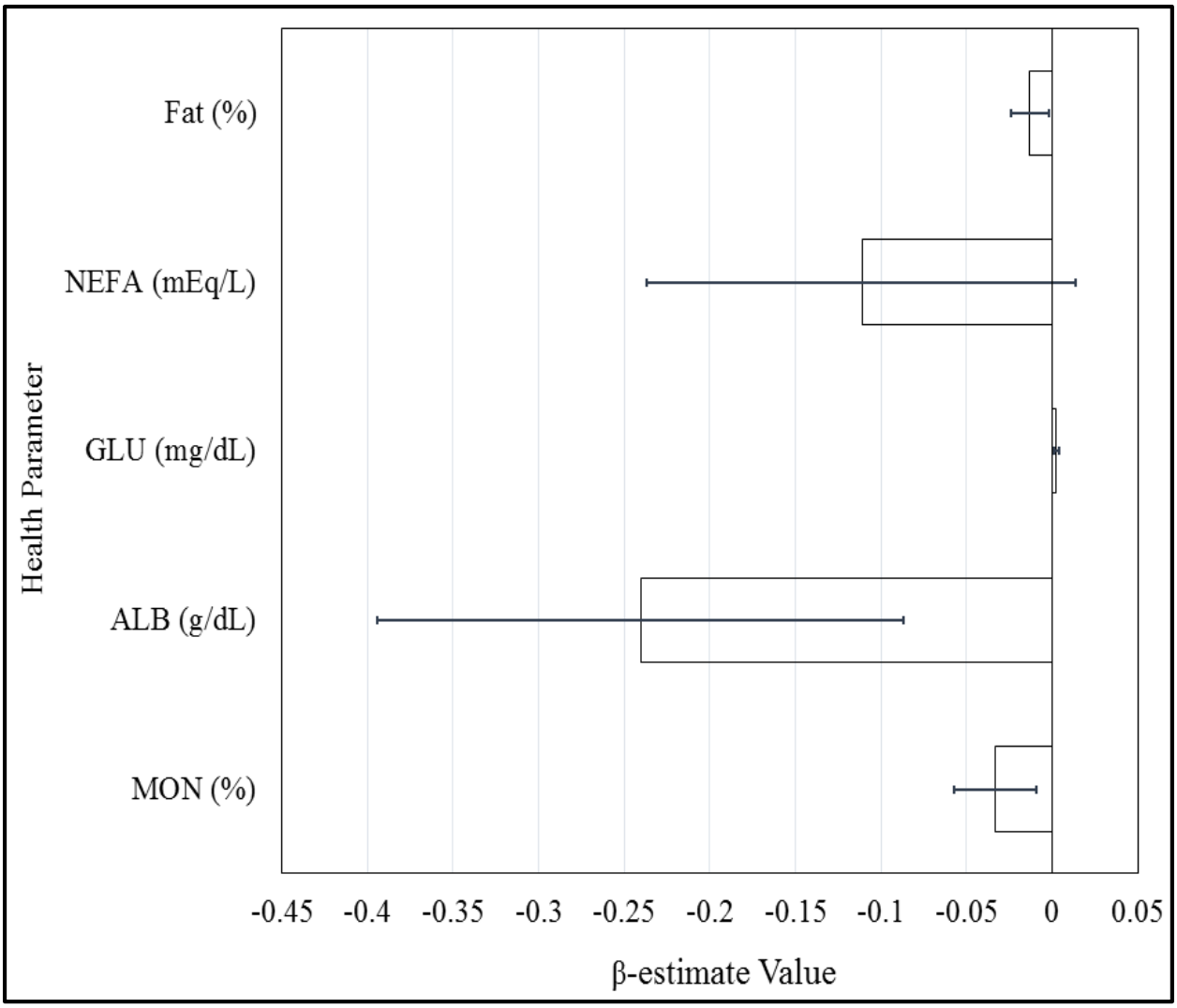

Figure 2.3. Beta-estimates (horizontal bars) and 85% confidence limits (capped solid lines) of Lesser Scaup (*Aythya affinis*) health parameters modeled as predictor variables of the Brillouin's species diversity index for intestinal helminths recovered from 130 female Lesser Scaup during their 2014 and 2015 spring migrations through the upper Midwest, USA. Health parameters from Lesser Scaup include: Fat = percent fat of Lesser Scaup body composition; NEFA = non-esterified fatty acids; GLU = glucose; ALB = albumin; MON = monocyte % of 100 cell white blood cell differential.

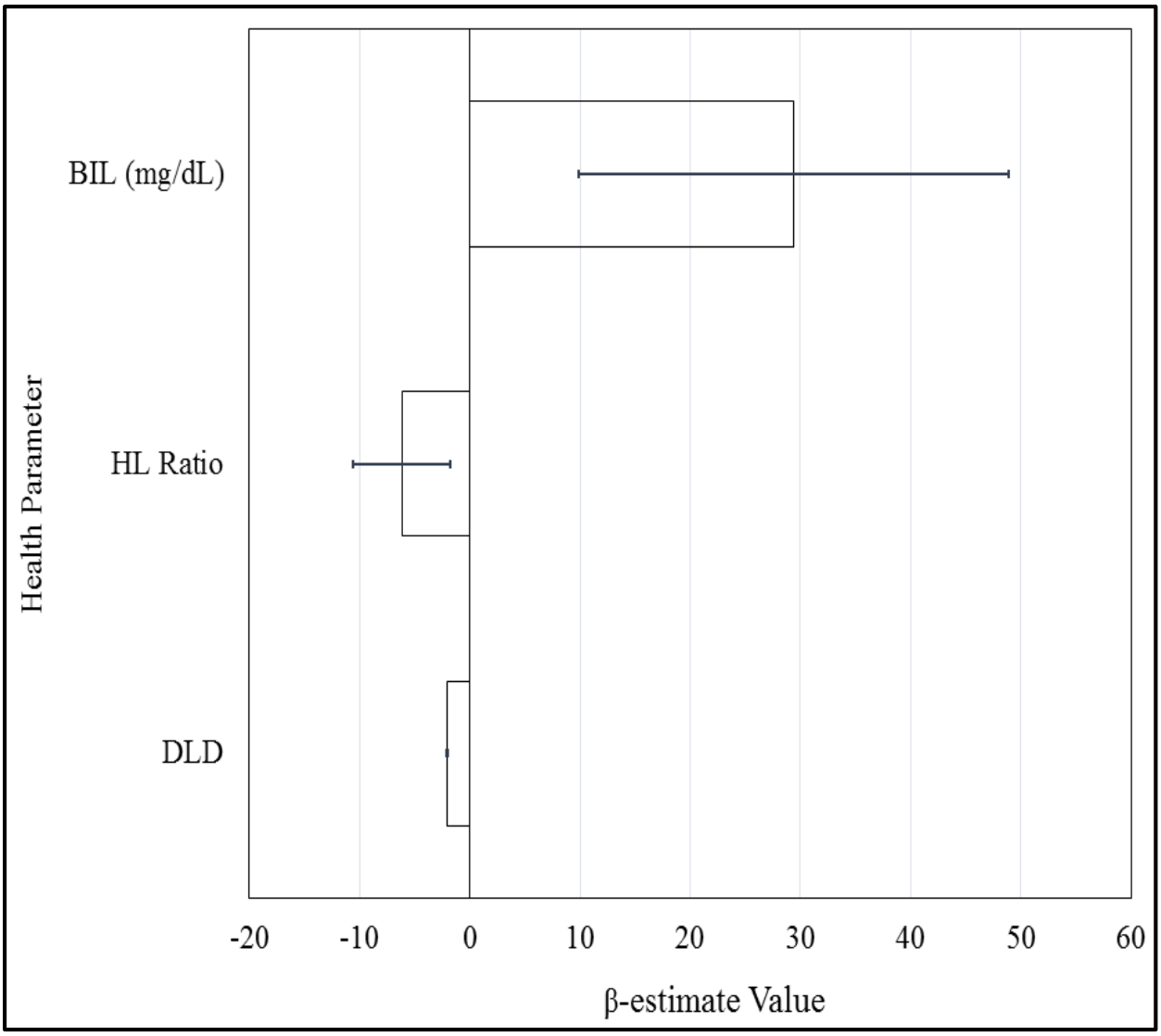

Figure 2.4. Back-transformed beta-estimates (horizontal bars) and 85% confidence limits (capped solid lines) of Lesser Scaup (*Aythya affinis*) health parameters modeled as predictor variables of the total cestode abundance. 130 female Lesser Scaup were collected during their 2014 and 2015 spring migrations through the upper Midwest, USA. Health parameters from Lesser Scaup include: BIL = bilirubin; HL Ratio = heterophil:lymphocyte ratio; DLD = daily lipid dynamics.

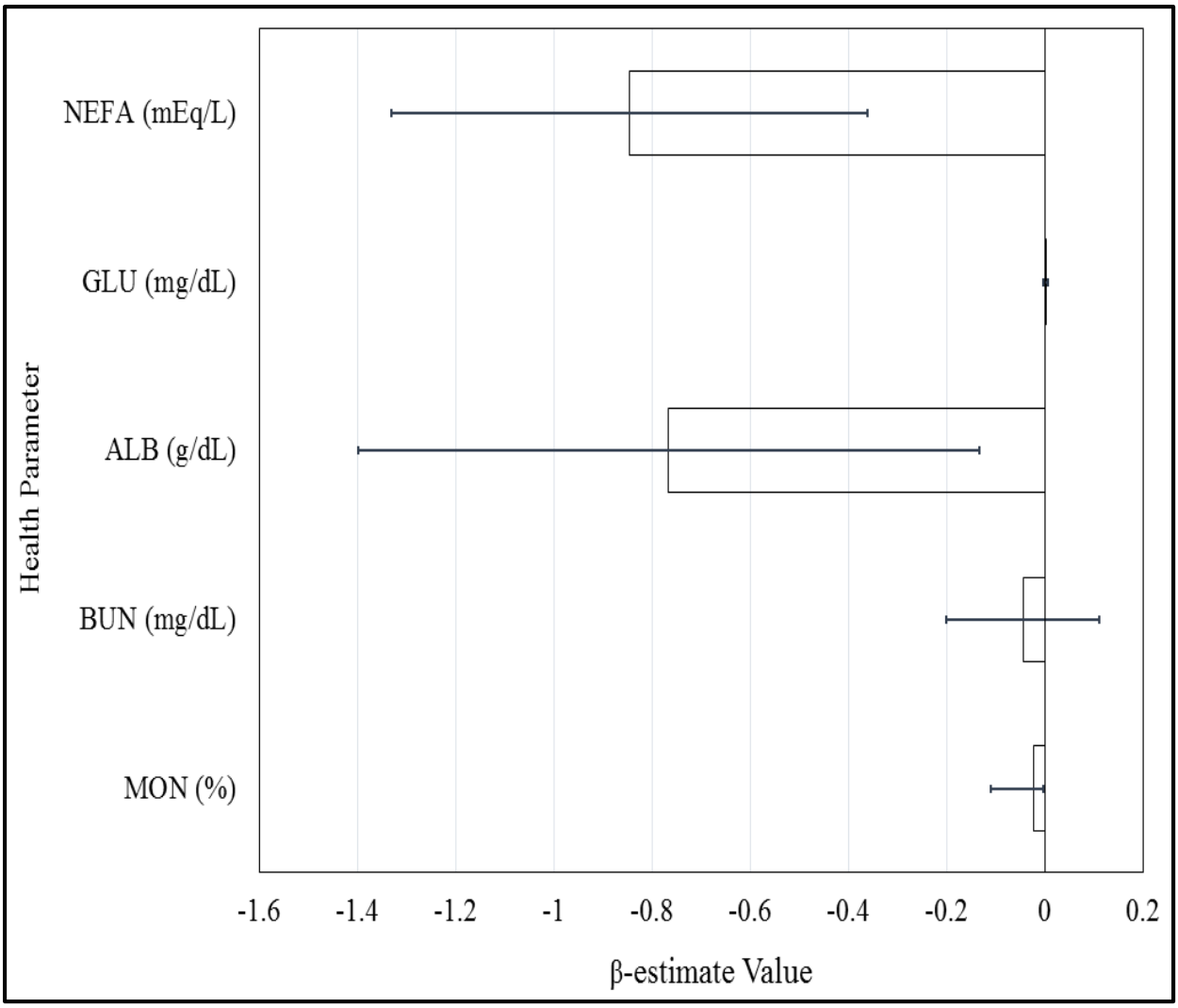

Figure 2.5. Beta-estimates (horizontal bars) and 85% confidence limits (capped solid lines) of Lesser Scaup (*Aythya affinis*) health parameters modeled as predictor variables of the total trematode abundance. 130 female Lesser Scaup were collected during their 2014 and 2015 spring migrations through the upper Midwest, USA. Health parameters from Lesser Scaup include: NEFA = non-esterified fatty acid; GLU = glucose; ALB = albumin; BUN = blood urea nitrogen; MON = monocyte % of 100 cell white blood cell differential.

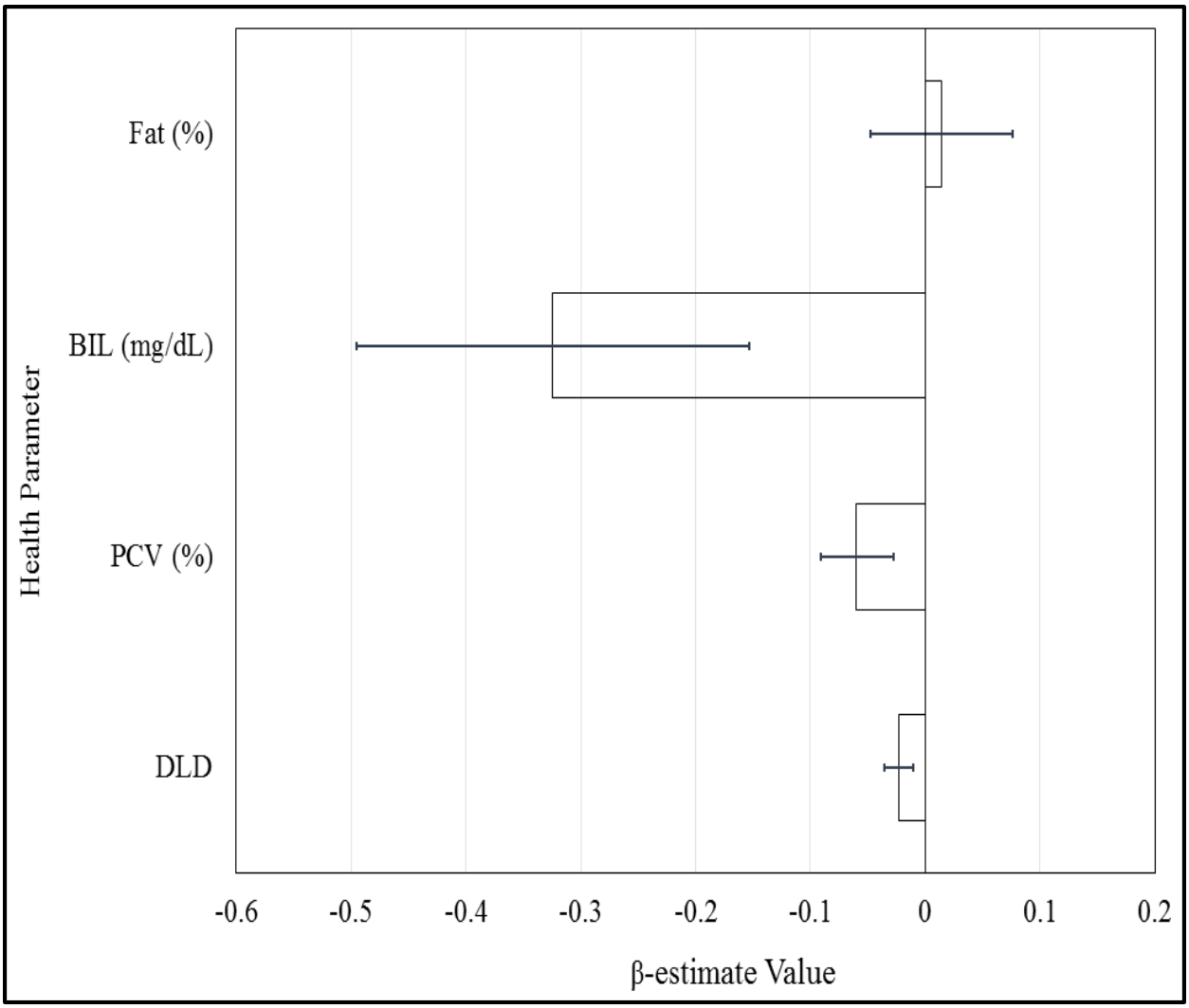

Figure 2.6. Beta-estimates (horizontal bars) and 85% confidence limits (capped solid lines) of Lesser Scaup (*Aythya affinis*) health parameters modeled as predictor variables of the intensity of the introduced trematode *Sphaeridiotrema* spp. infecting 39 female Lesser Scaup were collected during their 2014 and 2015 spring migrations through the upper Midwest, USA. Health parameters from Lesser Scaup include: Fat = percent fat of Lesser Scaup body composition; BIL = bilirubin (divided by 10); PCV = packed cell volume; DLD = daily lipid dynamics.

CHAPTER 3: SUMMARY

The continental decline of Lesser Scaup (*Aythya affinis*) has received much attention from wildlife researchers since the 1980's. Numerous factors have been suggested as contributing to the population wide decrease; however, a focal point of concern has been the association between food quantity and quality during spring migration, body condition, and subsequent reproductive success (Austin et al. 2006). The Mississippi Flyway encompasses many important stopover sites for Lesser Scaup (hereafter scaup) and hosts the majority of the continental population during migration (Bellrose 1976; Baldassarre 2014). Annual die-offs of scaup in the upper Midwest have recently elucidated the need for parasitological examinations investigating the dynamics between scaup health and intestinal helminth infection(s). Since 2002 along the Upper Mississippi River and surrounding regions, epizootic events caused by non-native trematodes have killed tens of thousands of waterbirds across several species, yet scaup seem to be the most impacted (Sauer et al. 2007). Although typically not considered as substantially influencing wild populations, parasites divert energy away from hosts and may therefore result in reduced host condition and fecundity (Wobeser 2008). Thus, parasitism may be considered as another factor contributing to the reduced condition of female scaup on the breeding grounds.

After the parasitological examinations of 130 female scaup, I enumerated and identified 647,174 helminths from 40 different taxa including 20 trematodes, 14 cestodes, 4 nematodes, and 2 acanthocephalans. Furthermore, I discovered that the intestinal helminth assemblages of scaup changed with time and geographic location, and these changes may be partially attributable to weather variations and food use. Age and body size were not associated with helminth infracommunity structure, but several helminth infracommunity parameters differed across the years and regions of my study area. Mean total abundance of helminths in 2015 was approximately a third of that found in 2014. The southernmost region of the study area, Rend Lake, had lower helminth diversity and abundance than collection sites in the Illinois River Valley (IRV), Central Mississippi River Valley (CMRV), and Upper Mississippi River Valley (UMRV). Similarly to total helminth abundance, mean total trematode abundance differed

between years of collection. Helminth species diversity and abundance generally increased with latitude and time.

A number of health parameters appeared as important predictors of helminth parameters. However, body fat and the blood metabolites non-esterified fatty acids, glucose, albumin, bilirubin, and blood urea nitrogen were associated with increased helminth loads. Additional hematologic parameters identified as influential were pack cell volume, the heterophil:lymphocyte ratio, basophil and monocyte white blood cell proportions. Lastly, the daily lipid dynamic (DLD), an index of habitat quality based on ratios of two blood metabolites, appeared in two model sets. Brillouin's species diversity index of helminths was most impacted by percent body fat, glucose, albumin, and monocytes. Total cestode abundance showed associations with bilirubin, the heterophil:lymphocyte ratio, and DLD measurements. Total nematode abundance was predicted by levels of bilirubin. Total trematode abundance was related to several relatively important predictor variables including glucose, blood urea nitrogen, and monocytes. Assessments of health parameter relationships with intensities of *Cyathocotyle bushiensis* and *Sphaeridiotrema* spp. were also conducted as part of this study, but the proportion of basophils was the only significantly important health parameter associated with *C. bushiensis* intensity. A packed cell volume, used to diagnose anemia, appeared as a moderately important predictor of *Sphaeridiotrema* spp. infections.

Baseline reference values were calculated for biochemical, hematological, hepatic elemental, diet composition, habitat quality, and morphometric data of female scaup collected in this study (Appendix). Mean, standard deviation (SD), and minimum-maximum values for all variables were calculated according to year, region, and scaup age. Additionally, values were again calculated for the pooled data of all 130 scaup. Helminth taxa prevalence and mean overall food availability (kg[dry]/ha) by region were also calculated and assessed to identify possible contributors influencing scaup condition. Variables that were not directly examined within statistical analyses were typically used to assist in identifying and/or supporting effects linked to scaup health. Several hepatic element concentrations were evaluated for

associations of scaup health and helminth infections, but were within ranges previously reported for scaup in the literature (Custer et al. 2003; Pillatzki 2007).

The materials composing scaup diets seemingly played a large part in intestinal helminth assemblage. Changes in diet are thought to induce intestinal helminth turnover by chemical (e.g., pH changes) and mechanical (e.g., abrasive scraping) methods during digestion (McLaughlin and Burt 1973). Invertebrate species composing scaup diets were most abundance and typically increased with latitude but were not important in predicting helminth infection parameters. Plant diet items where highest in the IRV and declined with latitude, whereas vertebrate food items were only evident in scaup collected from Rend Lake (Hagy et al. 2015). The vertebrate species found in scaup diets were entirely composed of American Gizzard Shad (*Dorosoma cepedianum*). Although these fish can serve as intermediate hosts for helminths infective to scaup, the fish observed being eaten by scaup were either moribund or dead due to the cold weather conditions experienced during the spring migration period.

While this project offers evidence for additional mechanisms underlying the Spring Condition Hypothesis (Anteau and Afton 2004) and shows a general decrease in scaup condition with increasing latitude (i.e., body mass, fat reserves, triglyceride deposition, packed cell volume, etc.), intestinal helminths are likely only a single component contributing to population declines. Effects of helminth infection(s) are probably additive in that intestinal helminth infections proliferate when a stressed individual's immune system can no longer meet the energetic demands of effective pathogen regulation (Wobeser 2008). For example, the plasma metabolites non-esterified fatty acids, albumin, and bilirubin accounted for the majority of summed weights across models and were each negatively associated with different helminth metrics. These metabolites have been previously associated with declining populations of various sea ducks due to the combined effects of habitat degradation and increased pathogenicity (Hollmén et al. 2001; Skerratt et al. 2005). Future research should investigate the underlying mechanisms involving these metabolites and various parameters of parasitic infections. The hematologic variables packed cell volume, monocytes, and basophils also appeared as relatively important predictors of several

helminth parameters, and relationships should be further investigated. I suggest that future research focus on controlled experimentation aimed at identifying individual health responses to various helminths and helminth infection parameters. Additionally, I advocate long-term studies examining scaup health across all periods of their annual cycle to further identify periods of conservation concern.

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APPENDIX

Table A.1. Mean (standard deviation) and minimum-maximum values of anatomical measurements by year of collection from 130 female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest, USA, during spring 2014 and 2015.

	2014			2015		
Anatomy[a]	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max
Mass (g)	60	538 (69.9)	400-680	70	550 (58.2)	460-740
Length (mm)	60	408 (9.3)	380-430	70	406 (8.7)	390-423
Keel (mm)	60	82.2 (4.1)	75-94	70	82 (3.7)	77-95
Wing (mm)	60	191 (5.3)	180-201	70	193 (4.3)	179-200
Tail (mm)	60	51 (3.7)	43-60	70	51 (3.0)	43-57
Culmen (mm)	60	40 (1.7)	36-44	70	40 (1.9)	35-48
Tarsus (mm)	60	40 (2.5)	34-45	70	41 (2.0)	36-46
Protein %	59	17.4 (1.29)	14.3-20.5	60	16.7 (1.36)	12.9-20.4
Fat %	59	15.8 (5.93)	2.6-24.8	60	13.8 (4.60)	4.2-24.5
Moisture %	59	59.5 (4.63)	52.1-70.1	60	63.1 (3.79)	54.9-70.6
Ash %	59	5.0 (0.67)	3.6-6.5	60	4.5 (1.15)	2.3-7.0

[a]Mass = eviscerated mass; length = entire body length; wing = wing chord length; protein, fat, moisture, ash = percent (%) body composition.

Table A.2. Mean (standard deviation) and minimum-maximum values of plasma and fecal biochemical analyte measurements by year of collection from 130 female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest, USA, during spring 2014 and 2015.

	2014			2015		
Analyte[a]	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max
BOHB (mmol/L)	56	0.67 (0.311)	0.10-1.77	68	0.63 (0.273)	0.20-1.60
NEFA (mEq/L)	58	0.702 (0.4298)	0.152-3.212	68	1.041 (0.4499)	0.343-2.424
TRIG (mg/dL)	58	233 (98.2)	73-523	67	267 (125.9)	50-650
BUN (mg/dL)	57	4 (1.5)	2-9	67	5 (1.5) B	3-9
GLU (mg/dL)	58	171 (39.1)	115-272	68	168 (56.6)	80-389
ALB (g/dL)	56	1.5 (0.33)	0.8-2.3	67	1.6 (0.43)	0.9-3.0
BIL (mg/dL)	58	0.3 (0.10)	0.1-0.5	68	0.4 (0.18) B	0.1-0.9
CORT (ng/mL)	41	7.03 (6.647)	0.35-42.93	65	5.36 (8.627)	0-48.56

[a]BOHB = β-hydroxybutyrate; NEFA = non-esterified fatty acids; TRIG = triglycerides; BUN = blood urea nitrogen; GLU = glucose; ALB = albumin; BIL = bilirubin; CORT = fecal corticosterone.

Table A.3. Mean (standard deviation) and minimum-maximum values of diet composition and habitat quality measurements by year of collection from 130 female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest, USA, during spring 2014 and 2015.

	2014			2015		
Parameter[a]	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max
DLD	58	-6.43 (25.629)	-52.12-65.65	68	-11.55 (22.913)	-54.90-77.74
Invert	60	3 (3.3)	0-15	70	2 (2.8)	0-12
Plant	60	3 (2.0)	0-8	70	1 (1.2)	0-5
Vert	60	-	-	70	0 (0.3)	0-1
Invert %	60	49.1 (45.36)	0-100	70	53.3 (47.47)	0-100
Plant %	60	44.3 (45.00)	0-100	70	13.8 (29.20)	0-100
Vert %	60	-	-	70	8.6 (25.60)	0-100

[a]DLD = daily lipid dynamic; Invert = count of invertebrate taxa per diet; Plant = count of plant taxa per diet; Vert = count of vertebrate taxa per diet; Invert % = aggregate invertebrate dry mass percent per diet; Plant % = aggregate plant dry mass percent per diet; Vert % = aggregate vertebrate dry mass percent per diet.

Table A.4. Mean (standard deviation) and minimum-maximum values of hepatic element concentration measurements by year of collection from 130 female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest, USA, during spring 2014 and 2015.

	2014			2015		
Elements (μg/g[dw])[a]	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max
As	60	0.29 (0.153)	0.02-0.84	60	0.25 (0.026)	0.22-0.28
Cd	60	1.876 (1.5811)	0.175-9.480	60	2.272 (3.6788)	0.090-16.783
Ca	60	174 (91.8)	77-593	60	139 (56.8)	87-440
Cr	60	0.50 (0.220)	0.01-1.01	60	0.17 (0.068)	0.11-0.32
Co	60	0.19 (0.086)	0.08-0.71	60	0.11 (0.097)	0.00-0.43
Cu	60	100.4 (35.91)	39.5-207.0	60	88.5 (30.00)	35.6-176.5
Fe	60	2229 (937.1)	299-5050	60	2245 (1166.8)	619-7060
Pb	60	0.158 (0.1724)	0.001-1.240	60	0.243 (0.3719)	0.023-2.404
Mg	60	749 (98.3)	529-1080	60	686 (64.9)	506-849
P	60	13101 (2208.6)	8980-20500	60	11022 (1175.2)	8159-13054
Se	60	16.4 (8.05)	5.5-46.7	60	12.5 (6.12)	4.4-42.6
Sr	60	0.079 (0.0756)	0.002-0.490	60	0.036 (0.0370)	0.023-0.266
Zn	60	151.0 (28.5)	105.0-261.0	60	119.0 (23.9)	49.2-188.8

[a]As = arsenic; Cd = cadmium; Ca = calcium; Cr = chromium; Co = cobalt; Cu = copper; Fe = iron; Pb = lead; Mg = magnesium; P = phosphorus; Se = selenium; Sr = strontium; Zn = zinc.

Table A.5. Mean (standard deviation) and minimum-maximum values of hematologic measurements by year of collection from 130 female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest, USA, during spring 2014 and 2015.

	2014			2015		
Hematology[a]	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max
PCV %	60	50.0 (9.51)	18.2-63.0	70	46.4 (11.28)	13.3-68.9
HET %	57	25 (11.3)	5-50	63	28 (11.5)	8-56
LYM %	57	66 (14.3)	36-95	63	65 (12.6)	36-88
HL Ratio	57	0.43 (0.274)	0.05-1.39	63	0.49 (0.294)	0.09-1.56
MON %	57	2 (2.2)	0-8	63	3 (2.6)	0-11
EOS %	57	1 (1.4)	0-6	63	1 (1.6)	0-8
BAS %	57	5 (4.8)	0-21	63	3 (3.0)	0-12

[a]PCV = packed cell volume; HET = heterophils; LYM = lymphocyte, HL ratio = heterophil:lymphocyte ratio; MON = monocytes; EOS = Eosinophils; BAS = Basophils.

Table A.6. Mean (standard deviation) and minimum-maximum values of intestinal helminth infracommunity parameter measurements by year of collection from 130 female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest, USA, during spring 2014 and 2015. Total abundance refers to all helminths found parasitizing Lesser Scaup. Total abundance refers to all helminth taxa, whereas total refers to the abundance for a specific taxon.

	2014			2015		
Helminth Parameter	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max
Species Richness	60	10 (3.5)	4-20	70	10 (4.1)	2-23
Brillouin's Diversity Index	60	1.18 (0.468)	0.06-2.02	70	1.09 (0.520)	0.15-2.10
Total Abundance	60	6997 (9418.0)	237-43391	70	3248 (4525.0)	26-19962
Acanthocephala Total	60	1 (4.7)	0-28	70	3 (16.2)	0-127
Cestoda Total	60	3401 (6157.8)	30-28648	70	2068 (3441.9)	1-15453
Trematoda Total	60	3565 (6688.8)	0-43178	70	1070 (1956.7)	0-9486
Nematoda Total	60	30 (59.2)	0-348	70	107 (675.8)	0-5674

Table A.7. Mean (standard deviation) and minimum-maximum values of anatomical measurements by region of collection from 130 female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest, USA, during spring 2014 and 2015. Regions were: Upper Mississippi River Valley (UMRV), Central Mississippi River Valley (CMRV), Illinois River Valley (IRV), and Southern Illinois (SI).

	SI[b]			IRV			CMRV			UMRV		
Anatomy[a]	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max
Mass (g)	10	526 (53.2)	460-620	60	541 (64.5)	400-700	22	571 (61.4)	480-680	38	539 (64.8)	400-740
Length (mm)	10	405 (6.0)	394-413	60	409 (9.4)	390-430	22	403 (9.3)	382-421	38	408 (8.2)	380-423
Keel (mm)	10	86 (5.6)	78-95	60	82 (4.0)	76-94	22	82 (2.9)	77-90	38	81 (3.0)	75-89
Wing (mm)	10	192 (3.2)	187-197	60	192 (5.2)	179-201	22	192 (4.4)	181-197	38	192 (5.1)	180-200
Tail (mm)	10	52 (1.8)	49-54	60	51 3.3)	44-60	22	50 (3.7)	43-55	38	52 (3.3)	43-58
Culmen (mm)	10	39 (1.8)	35-42	60	40 (2.1)	36-48	22	40 (1.5)	37-43	38	40 (1.2)	37-42
Tarsus (mm)	10	40 (1.2)	38-42	60	41 (2.4)	34-46	22	40 (2.6)	34-45	38	41 (2.4)	34-45
Protein %	0	-	-	59	17.3 (1.26)	14.8-20.5	22	16.8 (1.53)	12.9-20.4	38	16.8 (1.59)	14.3-19.8
Fat %	0	-	-	59	15.6 (5.43)	3.3-23.9	22	16 (5.70)	4.2-24.8	38	12.6 (5.19)	2.6-24.5
Moisture %	0	-	-	59	60.1 (4.80)	52.5-70.6	22	60.6 (4.62)	52.1-70.2	38	63.9 (3.68)	54.9-70.1
Ash %	0	-	-	59	4.8 (0.84)	2.8-6.5	22	4.7 (0.85)	2.3-5.9	38	4.6 (1.33)	2.4-7.0

[a]Mass = eviscerated mass; length = entire body length; wing = wing chord length; protein, fat, moisture, ash = percent (%) body composition.
[b]Proximate analyses of body composition were not conducted on Lesser Scaup collected from the SI region.

Table A.8. Mean (standard deviation) and minimum-maximum values of plasma and fecal biochemical analyte measurements by region of collection from 130 female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest, USA, during spring 2014 and 2015. Regions were: Upper Mississippi River Valley (UMRV), Central Mississippi River Valley (CMRV), Illinois River Valley (IRV), and Southern Illinois (SI).

	SI			IRV			CMRV			UMRV		
Analyte[a]	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max
BOHB (mmol/L)	9	0.84 (0.264)	0.46-1.27	56	0.65 (0.304)	0.10-1.77	22	0.61 (0.284)	0.24-1.13	37	0.61 (0.268)	0.15-1.24
NEFA (mEq/L)	9	1.050 (0.1378)	0.880-1.298	58	0.865 (0.3667)	0.326-2.361	22	0.928 (0.5811)	0.178-3.212	37	0.846 (0.5922)	0.152-2.424
TRIG (mg/dL)	8	333 (201.1)	100-650	58	241 (100.4)	50-523	22	269 (107.9)	120-549	37	235 (105.0)	82-519
BUN (mg/dL)	8	6 (2.0)	9-Mar	57	4 (1.3) A	8-Feb	22	5 (1.8)	9-Mar	37	5 (1.5)	8-Feb
GLU (mg/dL)	9	157 (59.0)	80-292	58	171 (42.9)	89-303	22	176 (55.1)	109-359	37	167 (53.3)	107-389
ALB (g/dL)	9	1.7 (0.56)	1.1-3.0	57	1.6 (0.27)	1.1-2.3	22	1.5 (0.44)	0.9-2.6	37	1.4 (0.44)	0.8-2.7
BIL (mg/dL)	9	0.4 (0.15)	0.2-0.7	58	0.3 (0.12)	0.1-0.7	22	0.3 (0.22)	0.1-0.8	37	0.3 (0.18)	0.1-0.9
CORT (ng/mL)	6	11.62 (14.096)	1.82-48.56	47	7.43 (8.492)	0.44-42.93	17	4.18 (3.130)	0.30-11.43	36	3.77 (4.867)	0-26.78

[a]BOHB = β-hydroxybutyrate; NEFA = non-esterified fatty acids; TRIG = triglycerides; BUN = blood urea nitrogen; GLU = glucose; ALB = albumin; BIL = bilirubin; CORT = fecal corticosterone.

Table A.9. Mean (standard deviation) and minimum-maximum values of diet composition and habitat quality measurements by region of collection from 130 female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest, USA, during spring 2014 and 2015. Regions were: Upper Mississippi River Valley (UMRV), Central Mississippi River Valley (CMRV), Illinois River Valley (IRV), and Southern Illinois (SI).

	SI			IRV			CMRV			UMRV		
Parameter[a]	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max
DLD	9	-6.71 (39.487)	-127.88	58	-8.06 (25.530)	-120.55	22	-7.54 (19.121)	-62.1	37	-12.56 (20.156)	-79.32
Invert	10	1 (0.9)	0-3	60	3 (2.9)	0-12	22	2 (1.9)	0-6	38	3 (3.8)	0-15
Plant	10	1 (0.8)	0-3	60	3 (2.1)	0-8	22	1 (1.2)	0-4	38	2 (1.8)	0-6
Vert	10	1 (0.4)	0-1	60	0	0	22	0	0	38	0	0
Invert %	10	23.1 (41.6)	0-100	60	46.4 (43.4)	0-100	22	55.7 (49.82)	0-100	38	64.1 (47.23)	0-100
Plant %	10	16.7 (26.3)	0-72.6	60	43.6 (43.1)	0-100	22	12.5 (32.25)	0-100	38	14.8 (34.13)	0-100
Vert %	10	60.2 (39.6)	0-100	60	0	0	22	0	0	38	0	0

[a]DLD = daily lipid dynamic; Invert = count of invertebrate taxa per diet; Plant = count of plant taxa per diet; Vert = count of vertebrate taxa per diet; Invert % = aggregate invertebrate dry mass percent per diet; Plant % = aggregate plant dry mass percent per diet; Vert % = aggregate vertebrate dry mass percent per diet.

Table A.10. Mean (standard deviation) and minimum-maximum values of hepatic element concentration measurements by region of collection from 130 female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest, USA, during spring 2014 and 2015. Regions were: Upper Mississippi River Valley (UMRV), Central Mississippi River Valley (CMRV), Illinois River Valley (IRV), and Southern Illinois (SI).

	SI[b]			IRV			CMRV			UMRV		
Element (μg/g[dw])[a]	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max
As	0	-	-	60	0.29 (0.134)	0.025-0.840	22	0.26 (0.088)	0.02-0.46	38	0.23 (0.068)	0.02-0.28
Cd	0	-	-	60	2.231 (2.6020)	0.120-16.783	22	1.991 (3.6729)	0.160-16.017	38	1.921 (3.2925)	0.090-16.317
Ca	0	-	-	60	151 (77.8)	77-593	22	172 (96.0)	90-440	38	151 (73.4)	87-525
Cr	0	-	-	60	0.37 (0.253)	0.01-0.98	22	0.27 (0.181)	0.11-0.62	38	0.27 (0.229)	0.11-1.01
Co	0	-	-	60	0.174 (0.1039)	0.003-0.710	22	0.153 (0.0848)	0.003-0.333	38	0.108 (0.1058)	0.003-0.433
Cu	0	-	-	60	92.9 (36.83)	39.5-207.0	22	102.5 (33.61)	53.9-177.0	38	90.7 (31.62)	35.6-176.5
Fe	0	-	-	60	2306 (1114.5)	299-5162	22	2347 (862.7)	706-3763	38	2067 (1221.4)	619-7060
Pb	0	-	-	60	0.217 (0.3727)	0.001-2.404	22	0.220 (0.1571)	0.067-0.689	38	0.174 (0.2737)	0.023-1.185
Mg	0	-	-	60	704 (88.8)	506-1080	22	711 (68.2)	556-834	38	734 (104.8)	539-1030
P	0	-	-	60	11962 (2074.7)	8159-20500	22	12188 (1891.2)	8958-15900	38	11893 (2280.5)	8325-18400
Se	0	-	-	60	15.12 (7.225)	5.13-34.30	22	15.39 (8.8638)	4.8-46.7	38	12.30 (7.280)	4.43-42.62
Sr	0	-	-	60	0.062 (0.0722)	0.002-0.490	22	0.054 (0.0655)	0.002-0.266	38	0.046 (0.0493)	0.023-0.280
Zn	0	-	-	60	136.0 (31.43)	49.2-217.0	22	132.5 (25.95)	75.9-188.1	38	130.7 (35.56)	73.6-261.0

[a]As = arsenic; Cd = cadmium; Ca = calcium; Cr = chromium; Co = cobalt; Cu = copper; Fe = iron; Pb = lead; Mg = magnesium; P = phosphorus; Se = selenium; Sr = strontium; Zn = zinc.

[b]Hepatic element analyses were not conducted on Lesser Scaup collected from the SI region.

Table A.11. Mean (standard deviation) and minimum-maximum values of hematologic measurements by region of collection from 130 female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest, USA, during spring 2014 and 2015. Regions were: Upper Mississippi River Valley (UMRV), Central Mississippi River Valley (CMRV), Illinois River Valley (IRV), and Southern Illinois (SI).

	SI			IRV			CMRV			UMRV		
Hematology[a]	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max
PCV %	10	42.7 (12.99)	25.6-67.2	60	49.6 (8.71)	32.7-68.9	22	50.0 (10.53)	13.3-67.9	38	44.3 (11.65)	18.2-67.3
HET %	9	29 (11.3)	12-46	56	27 (12.2)	5-50	21	22 (7.8)	9-39	34	29 (11.6)	12-56
LYM %	9	64 (11.8)	46-80	56	63 (14.3)	36-95	21	72 (10.0)	54-90	34	65 (13.1)	36-88
HL Ratio	9	0.49 (0.258)	0.15-1.02	56	0.48 (0.302)	0.05-1.39	21	0.32 (0.154)	0.10-0.72	34	0.50 (0.309)	0.14-1.56
MON %	9	3 (2.5)	0-9	56	2 (2.5)	0-10	21	3 (2.2)	0-7	34	2 (2.6)	0-11
EOS %	9	2 (1.4)	0-4	56	1 (1.4)	0-6	21	2 (1.8)	0-6	34	1 (1.6)	0-8
BAS %	9	3 (3.3)	0-10	56	5 (4.3)	0-19	21	2 (2.7)	0-8	34	2 (3.7)	0-21

[a]PCV = packed cell volume; HET = heterophils; LYM = lymphocyte, HL ratio = heterophil:lymphocyte ratio; MON = monocytes; EOS = Eosinophils; BAS = Basophils.

Table A.12. Mean (standard deviation) and minimum-maximum values of intestinal helminth infracommunity parameter measurements by region of collection from 130 female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest, USA, during spring 2014 and 2015. Regions were: Upper Mississippi River Valley (UMRV), Central Mississippi River Valley (CMRV), Illinois River Valley (IRV), and Southern Illinois (SI). Total abundance refers to all helminth taxa, whereas total refers to the abundance for a specific taxon.

	SI			IRV			CMRV			UMRV	
Parameter	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max
Species Richness	10	7 (2.5)	2-11	60	10 (3.4)	4-19	22	10 (5.0)	3-23	38	11 (3.4)	3-18
Brillouin's Diversity Index	10	0.81 (0.386)	0.21-1.36	60	1.13 (0.439)	0.32-2.05	22	0.93 (0.543)	0.06-1.92	38	1.34 (0.500)	0.16-2.10
Total Abundance	10	832 (669.0)	26-1746	60	5212 (6936.9)	125-30316	22	7012 (9868.6)	91-43391	38	4523 (7237.1)	83-38949
Acanthocephala Total	10	1 (0.6)	0-2	60	2 (7.8)	0-49	22	6 (27.1)	0-127	38	-	-
Cestoda Total	10	714 (668.8)	10-1739	60	3358 (5734.3)	24-28648	22	2608 (3823.1)	2-12520	38	2180 (4606.9)	1-25491
Trematoda Total	10	100 (278.7)	0-892	60	1823 (3482.9)	0-24441	22	4367 (9236.7)	0-43178	38	2167 (3452.1)	0-13457
Nematoda Total	10	19 (20.7)	5-72	60	28 (44.2)	0-270	22	31 (44.0)	0-148	38	177 (917.6)	0-5674

Table A.13. Mean (standard deviation) and minimum-maximum morphometric measurements by age class (after hatch year [AHY] and hatch year [HY]) of 130 female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest, USA, during spring 2014 and 2015.

	AHY			HY		
Anatomy[a]	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max
Mass (g)	110	549 (62.1)	420-740	20	519 (68.9)	400-640
Length (mm)	110	408 (8.7)	382-430	20	402 (9.1)	380-419
Keel (mm)	110	82 (3.8)	76-95	20	83 (4.4)	75-91
Wing (mm)	110	192 (5.0)	179-201	20	191 (4.3)	181-197
Tail (mm)	110	51 (3.3)	43-59	20	51 (3.7)	43-60
Culmen (mm)	110	40 (1.9)	35-48	20	39 (1.4)	37-42
Tarsus (mm)	110	41 (2.3)	34-46	20	40 (2.8)	34-45
Protein %	104	17.0 (1.36)	12.9-20.5	15	17.6 (1.36)	15.6-20.4
Fat %	104	14.8 (5.39)	2.6-24.8	15	14.0 (5.08)	3.7-23.1
Moisture %	104	61.5 (4.68)	52.1-70.6	15	61.0 (3.79)	52.9-69.5
Ash %	104	4.7 (1.02)	2.3-7.0	15	5.0 (0.63)	4.1-6.5

[a]Mass = eviscerated mass; length = entire body length; wing = wing chord length; protein, fat, moisture, ash = percent (%) body composition.

Table A.14. Mean (standard deviation) and minimum-maximum plasma and fecal biochemical analyte measurements by age class (after hatch year [AHY] and hatch year [HY]) of 130 female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest, USA, during spring 2014 and 2015.

	AHY			HY		
Analyte[a]	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max
BOHB (mmol/L)	105	0.63 (0.282)	0.14-1.77	19	0.77 (0.311) B	0.10-1.28
NEFA (mEq/L)	107	0.873 (0.4794)	0.152-3.212	19	0.946 (0.4246)	0.178-2.167
TRIG (mg/dL)	106	252 (115.2)	50-650	19	249 (115.8)	100-453
BUN (mg/dL)	106	5 (1.6)	0.8-2.7	19	5 (1.2)	3.0-7.0
GLU (mg/dL)	107	172 (50.6)	89-389	19	157 (39.1)	80-264
ALB (g/dL)	104	1.5 (0.38)	0.8-2.7	19	1.6 (0.43)	1.0-3.0
BIL (mg/dL)	107	0.3 (0.16)	0.1-0.9	19	0.3 (0.16)	0.1-0.8
CORT (ng/mL)	91	5.88 (7.781)	0-48.56	15	7.49 (7.884)	0.38-32.92

[a]BOHB = β-hydroxybutyrate; NEFA = non-esterified fatty acids; TRIG = triglycerides; BUN = blood urea nitrogen; GLU = glucose; ALB = albumin; BIL = bilirubin; CORT = fecal corticosterone.

Table A.15. Mean (standard deviation) and minimum-maximum diet composition and habitat quality measurements by age class (after hatch year [AHY] and hatch year [HY]) of 130 female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest, USA, during spring 2014 and 2015.

	AHY			HY		
Parameter[a]	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max
DLD	107	-8.041 (23.5521)	-54.902-77.744	19	-15.481 (27.5560)	-50.139-65.648
Invert	110	3 (2.9)	0-12	20	2 (3.7)	0-15
Plant	110	2 (2.0)	0-8	20	2 (1.6)	0-5
Vert	110	0 (0.2)	0-1	20	0 (0.4)	0-1
Invert %	110	52.9 (46.01)	0-100	20	42.9 (48.70)	0-100
Plant %	110	28.5 (40.47)	0-100	20	24.2 (39.44)	0-100
Vert %	110	2.2 (12.59)	0-100	20	17.9 (37.12)	0-100

[a]DLD = daily lipid dynamic; Invert = count of invertebrate taxa per diet; Plant = count of plant taxa per diet; Vert = count of vertebrate taxa per diet; Invert % = aggregate invertebrate dry mass percent per diet; Plant % = aggregate plant dry mass percent per diet; Vert % = aggregate vertebrate dry mass percent per diet.

Table A.16. Mean (standard deviation) and minimum-maximum hepatic element concentration measurements by age class (after hatch year [AHY] and hatch year [HY]) of 130 female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest, USA, during spring 2014 and 2015.

	AHY			HY		
Element (μg/g[dw])[a]	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max
As	105	0.27 (0.113)	0.02-0.84	15	0.24 (0.065)	0.02-0.37
Cd	105	2.114 (2.8084)	0.090-16.783	15	1.953 (3.4504)	0.120-16.017
Ca	105	155 (78.5)	77-593	15	155 (67.7)	107-413
Cr	105	0.31 (0.223)	0.01-1.01	15	0.38 (0.253)	0.11-0.96
Co	105	0.149 (0.1051)	0.003-0.710	15	0.150 (0.0728)	0.003-0.333
Cu	105	93.3 (33.89)	35.6-207.0	15	97.9 (30.14)	48.3-177.0
Fe	105	2208 (1128.7)	299-7060	15	2401 (574.7)	1460-3563
Pb	105	0.204 (0.3192)	0.001-2.404	15	0.205 (0.1501)	0.064-0.689
Mg	105	711 (92.2)	506-1080	15	737 (52.2)	623-831
P	105	11825 (2031.7)	8159-20500	15	12842 (1706.3)	10823-15900
Se	105	14.60 (7.716)	4.43-46.70	15	12.51 (4.268)	5.76-23.40
Sr	105	0.055 (0.0634)	0.002-0.490	15	0.059 (0.0540)	0.002-0.266
Zn	105	133.9 (32.63)	49.2-261.0	15	132.4 (14.24)	100.9-156.0

[a]As = arsenic; Cd = cadmium; Ca = calcium; Cr = chromium; Co = cobalt; Cu = copper; Fe = iron; Pb = lead; Mg = magnesium; P = phosphorus; Se = selenium; Sr = strontium; Zn = zinc.

Table A.17. Mean (standard deviation) and minimum-maximum hematologic measurements by age class (after hatch year [AHY] and hatch year [HY]) of 130 female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest, USA, during spring 2014 and 2015.

	AHY			HY		
Hematology[a]	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max
PCV %	110	48.1 (10.34)	18.2-68.9	20	45.0 (11.54)	13.3-60.9
HET %	101	27 (11.9)	5-56	19	26 (9.3)	9-40
LYM %	101	65 (13.7)	36-95	19	65 (12.2)	40-90
HL Ratio	101	0.47 (0.300)	0.05-1.56	19	0.42 (0.183)	0.10-0.73
MON %	101	2 (2.5)	0-11	19	3 (2.1)	0-9
EOS %	101	1 (1.5)	0-8	19	1 (1.8)	0-6
BAS %	101	4 (4.2)	0-21	19	3 (3.3)	0-10

[a]PCV = packed cell volume; HET = heterophils; LYM = lymphocyte, HL ratio = heterophil:lymphocyte ratio; MON = monocytes; EOS = Eosinophils; BAS = Basophils.

Table A.18. Mean (standard deviation) and minimum-maximum intestinal helminth infracommunity parameter measurements by age class (after hatch year [AHY] and hatch year [HY]) of 130 female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest, USA, during spring 2014 and 2015. Total abundance refers to all helminth taxa, whereas total refers to the abundance for a specific taxon.

	AHY			HY		
Parameter	*n*	Mean (SD)	Min-Max	*n*	Mean (SD)	Min-Max
Species Richness	110	10 (3.6)	3-19	20	10 (5.0)	2-23
Brillouin's Diversity Index	110	1.14 (0.491)	0.06-2.10	20	1.15 (0.543)	0.21-1.92
Total Abundance	110	5069 (7690.4)	26-433391	20	4478 (5839.6)	40-19962
Acanthocephala Total	110	2 (13.3)	0-127	20	1 (2.5)	0-11
Cestoda Total	110	2760 (5163.9)	1-28648	20	2261 (3255.1)	0-10414
Trematoda Total	110	2228 (5145.5)	0-43178	20	2188 (3395.5)	0-11244
Nematoda Total	110	79 (540.5)	0-5674	20	29 (39.0)	0-148

Table A.19. Mean (standard deviation) and minimum-maximum values of anatomical measurements from all female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest, USA, during spring 2014 and 2015.

Anatomy[a]	*n*	Mean (SD)	Min-Max
Mass (g)	130	545 (63.9)	400-740
Length (mm)	130	407 (9.0)	380-430
Keel (mm)	130	82 (3.9)	75-95
Wing (mm)	130	192 (4.9)	179-201
Tail (mm)	130	51 (3.3)	43-60
Culmen (mm)	130	40 (1.8)	35-48
Tarsus (mm)	130	41 (2.4)	34-46
Protein %	119	17.1 (1.37)	12.9-20.5
Fat %	119	14.7 (5.33)	2.6-24.8
Moisture %	119	61.4 (5.54)	52.1-70.6
Ash %	119	4.7 (0.98)	2.3-7.0

[a]Mass = eviscerated mass; length = entire body length; wing = wing chord length; protein, fat, moisture, ash = percent (%) body composition.

Table A.20. Mean (standard deviation) and minimum-maximum values of plasma and fecal biochemical analyte measurements from all female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest, USA, during spring 2014 and 2015.

Analyte[a]	*n*	Mean (SD)	Min-Max
BOHB (mmol/L)	124	0.65 (0.290)	0.10-1.77
NEFA (mEq/L)	126	0.884 (0.4706)	0.152-3.212
TRIG (mg/dL)	125	251 (114.8)	50-650
BUN (mg/dL)	124	5 (1.5)	2-9
GLU (mg/dL)	126	170 (49.2)	80-389
ALB (g/dL)	123	1.5 (0.39)	0.8-3.0
BIL (mg/dL)	126	0.3 (0.16)	0.1-0.9
CORT (ng/mL)	106	6.13 (7.788)	0-48.56

[a]BOHB = β-hydroxybutyrate; NEFA = non-esterified fatty acids; TRIG = triglycerides; BUN = blood urea nitrogen; GLU = glucose; ALB = albumin; BIL = bilirubin; CORT = fecal corticosterone.

Table A.21. Mean (standard deviation) and minimum-maximum values of habitat quality measures from localities of collection for all female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest, USA, during spring 2014 and 2015.

Parameter[a]	*n*	Mean (SD)	Min-Max
DLD	126	-9.185 (24.2447)	-54.902-77.744
Invert	130	3 (3.0)	0-15
Plant	130	2 (1.9)	0-8
Vert	130	0 (0.2)	0-1
Invert %	130	51.4 (46.38)	0-100
Plant %	130	27.9 (40.19)	0-100
Vert %	130	4.6 (19.21)	0-100

[a]DLD = daily lipid dynamic; Invert = count of invertebrate taxa per diet; Plant = count of plant taxa per diet; Vert = count of vertebrate taxa per diet; Invert % = aggregate invertebrate dry mass percent per diet; Plant % = aggregate plant dry mass percent per diet; Vert % = aggregate vertebrate dry mass percent per diet.

Table A.22. Mean (standard deviation) and minimum-maximum values of hepatic element concentrations from all female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest, USA, during spring 2014 and 2015.

Elements (μg/g[dw])[a]	*n*	Mean (SD)	Min-Max
As	120	0.26 (0.107)	0.02-0.84
Cd	120	2.089 (2.9020)	0.090-16.783
Ca	120	155 (76.5)	77-593
Cr	120	0.32 (0.228)	0.01-1.01
Co	120	0.149 (0.1005)	0.003-0.710
Cu	120	94.0 (33.27)	35.6-207.0
Fe	120	2238 (1063.0)	299-7060
Pb	120	0.204 (0.2990)	0.001-2.404
Mg	120	715 (87.6)	506-1080
P	120	11982 (2013.0)	8159-20500
Se	120	14.28 (7.319)	4.43-46.70
Sr	120	0.056 (0.0618)	0.002-0.490
Zn	120	133.7 (30.49)	49.2-261.0

[a]As = arsenic; Cd = cadmium; Ca = calcium; Cr = chromium; Co = cobalt; Cu = copper; Fe = iron; Pb = lead; Mg = magnesium; P = phosphorus; Se = selenium; Sr = strontium; Zn = zinc.

Table A.23. Mean (standard deviation) and minimum-maximum values of hematologic measurements from all female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest, USA, during spring 2014 and 2015.

Hematology[a]	*n*	Mean (SD)	Min-Max
PCV %	130	47.6 (10.54)	13.3-68.9
HET %	120	27 (11.5)	5-56
LYM %	120	65 (13.4)	36-95
HL Ratio	120	0.46 (0.286)	0.05-1.56
MON %	120	2 (2.5)	0-11
EOS %	120	1 (1.5)	0-8
BAS %	120	4 (4.0)	0-21

[a]PCV = packed cell volume; HET = heterophils; LYM = lymphocyte, HL ratio = heterophil:lymphocyte ratio; MON = monocytes; EOS = Eosinophils; BAS = Basophils.

Table A.24. Mean (standard deviation) and minimum-maximum values of intestinal helminth infracommunity parameters from 130 female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest, USA, during spring 2014 and 2015. Total abundance refers to all helminth taxa, whereas total refers to the abundance for a specific taxon.

Helminth Parameter	*n*	Mean (SD)	Min-Max
Species Richness	130	10 (3.8)	2-23
Brillouin's Diversity Index	130	1.13 (0.497)	0.06-2.10
Total Abundance	130	4978 (7419.0)	0-43391
Acanthocephala Total	130	2 (12.3)	0-127
Cestoda Total	130	2683 (4911.7)	1-28648
Trematoda Total	130	2222 (4906.1)	0-43178
Nematoda Total	130	71 (497.4)	0-5674

Table A.25. Mean (standard error) and minimum-maximum values of available biomass (kg[dry]/ha) of combined invertebrate, plant, and vertebrate food types by location within region of the study area where 130 female Lesser Scaup (*Aythya affinis*) collected from four regions of the upper Midwest, USA, during spring 2014 and 2015. Regions were: Upper Mississippi River Valley (UMRV), Central Mississippi River Valley (CMRV), Illinois River Valley (IRV), and Southern Illinois (SI).

Region		*n*	Mean (SE)	Min-Max
UMRV		**59**	**235.2 (69.56)**	**11.53-1563.92**
	Pool 7	22	273.9 (81.58)	15.42-1563.92
	Pool 8	19	129.4 (56.17)	11.53-1005.81
	Pool 9	18	256.0 (63.77)	11.89-919.79
CMRV		**52**	**100.4 (39.40)**	**3.27-900.02**
	Pool 12	21	117.9 (39.40)	4.77-670.84
	Pool 19	31	88.53 (42.47)	3.27-900.02
IRV		**174**	**340.7 (119.56)**	**1.08-5235.13**
	Peoria Reach	29	278.2 (60.64)	4.62-929.56
	La Grange Reach	125	359.5 (129.06)	6.90-5235.13
	Alton Reach	20	313.6 (120.40)	1.08-2061.70
SI		**10**	**14.2 (2.03)**	**3.38-31.48**
	Rend Lake	10	14.2 (2.03)	3.38-31.48